HORMONES

A SURVEY OF THEIR PROPERTIES AND USES

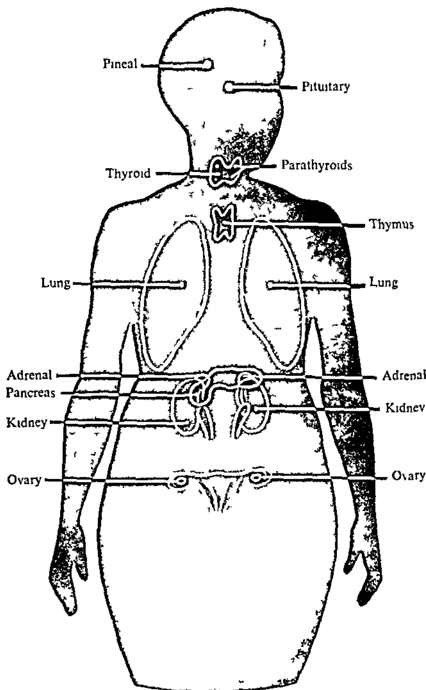


Fig 1

POSITION OF ENDOCRINE GLANDS IN THE FEMALE

The pineal and thymus glands are included in the diagram although their functions are still obscure The lungs and kidneys are shown to indicate their position relative to the glands

HORMONES:

A SURVEY OF THEIR PROPERTIES AND USES

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PREFACE

The chemical messengers however or hormones (from ορμαω I excite or arouse) as we might call them E H STARLING M D F R C P F R S The Croonian Lectures 1905

In the forty six years since they were so named by Starling the number of substances now recognised as hormones has increased con siderably and the available information is far too extensive to be in cluded in one volume In selecting the material for this book the aim has been to provide pharmacists medical practitioners and students with an account of those hormones and endocrine glands which have well defined pharmacological effects and therapeutic applications to gether with information on closely related substances such as the artificial hormones and antihormones it is for this reason that the pineal and thymus glands and a number of hormones including secre tin have been omitted It should be noted that the term artificial hormone is preferred to synthetic hormone for denoting those sub stances with hormonal activity which are produced solely in the laboratory since many naturally occurring hormones can now be com pletely synthesised Many original papers are quoted in the text references to which are give at the end of each chapter These together with an extensive bibliography should be of value to laboratory workers and others who may wish to obtain further details of particular aspects of the subject

Beginning with an historical account of the development of the science of endocrinology from early work with gland extracts to the isolation and synthesis of active principles the book continues with an account of the physiology of the glands in which the normal function and the effects of dysfunction of each gland are described and the importance and limitations of replacement therapy outlined

The chemistry of the hormones may conveniently be divided into two sections Those hormones having a steroid nucleus form a group of closely related and well defined substances whereas the non steroid hormones are chemically of a heterogeneous nature having a variety of structures Such an arrangement has been adopted in this book In addition the chapter on the chemistry of the steroid hormones opens with a brief account of the general chemistry of steroids to serve as a basis for explaining the more detailed chemistry of the hormones themselves It also includes an account of the artificial hormones since these are related chemically or physiologically to certain of the steroid hormones

As more and more hormones are isolated and characterised so bio logical methods of assay tend to be replaced by chemical methods

Nevertheless there are still many hormones which are assayed biologically and in the chapter on standardisation there is a full account of the principles underlying the application of biological methods and descriptions of the more important assays together with an explanation of the various units which are or have been used to express activity

The chapter on action and uses deals with the pharmacology and therapeutics of the glands and their hormones and of the artificial hormones and antihormones It indicates the various forms in which the substances are administered and also describes the method of administration by implantation Preparations referred to in this chapter are described more fully in the next which deals with the pharmacy of hormones and related substances and their preparations Methods and formulæ are given for a variety of preparations including injections solutions ointments creams and suppositories Methods of sterilisation conditions for storage and the requirements of the Therapeutic Substances Act 1925 and Regulations made thereunder and of the Pharmacy and Poisons Act 1933 in so far as they apply to glands and hormones are also described The final chapter gives details of the commercial forms of many of the preparations referred to in other parts of the book and the names and addresses of the manufacturers

The abbreviations used for weights and measures are in accordance with those of the British Pharmacopœia 1948 and temperatures are expressed in degrees centigrade

The Council of the Pharmaceutical Society is indebted to the following contributors for preparing the chapters indicated H E Dale (Physiology and Action and Uses) C W Emmens D Sc Ph D (Standardisation) D H Hey D Sc Ph D F R I C (Chemistry) and T D Whittet Ph C D B A (Pharmacy) It is also indebted to P M F Bishop D M J F Wilkinson M D F R C P and P R Evans F R C P M D for clinical photographs and advice and to the chief pharmacists of many hospitals for certain pharmaceutical formulæ

June
1951

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INTRODUCTION

FOR many centuries glands have been known to anatomists but it is only within comparatively recent times that they have been recognised as organs which have the property of secreting chemical substances of great biological importance Those which secrete directly into the blood stream are known as ductless glands endocrine glands or organs of internal secretion and the substances which they secrete are called hormones The importance of these organs began to be appreciated about the middle of the last century when it was found that destruction of the adrenal glands had far reaching effects in remote parts of the body Endocrinology is the branch of science which deals with the functions and nature of these organs and their secretions The chemical constitution of some hormones is known and many have been synthesised in the laboratory other hormones resemble proteins and their exact chemical constitution is not yet known

It is characteristic of the hormones that only minute quantities are required to produce profound physiological reactions in the body The hormone of the adrenal medulla known as adrenaline is highly active when present in the blood in a concentration of les than one part in one hundred million All the endocrine glands are characterised by having a plentiful supply of blood which can carry the hormones rapidly to all parts of the body The main endocrine organs and their position in the human body are shown diagrammatically in the frontispiece The hormones associated with each gland are listed in Table I

The Ductless Glands The thyroid is found in all vertebrate animals In man it takes the form of a pair of lobes situated on each side of the trachea at the base of the neck with a connecting band of tissue known as the isthmus In the adult the gland weighs about twenty five grammes and its structure consi ts of a large number of vesicles lined with secreting cells and filled with a jelly like colloid In conditions of over activity the microscopic structure undergoes a fundamental change The hormone of the thyroid is the amino acid thyroxine 3 5-diiodotyrosine is also found in the gland but this is regarded as a precursor in the biogenesis of the hormone Both compounds have been synthesised The secretions of the thyroid gland have a marked effect on the basal metabolic rate Excessive activity of the gland speeds up all the vital processes causes acute nervous tension and may result in exophthalmic goitre A deficiency of the hormone in the adult results in the condition known as myxœdema which is characterised by a sluggish temperament and appearance and in children a congenital lack of the hormone results in cretinism

The parathyroids are small yellowish red organs which generally occur in pairs They vary in size number and position but in man

they are usually found on the inner side and towards the back of each lobe of the thyroid gland The hormone appears to be a protein but little is known about its constitution Its main function is to control the metabolism of calcium and inorganic phosphates

TABLE I

ENDOCRINE GLANDS AND THEIR HORMONES

Gland	*Hormones*
Thyroid	Thyroxine
Parathyroid	A protein (not yet identified)
Pancreas	
Islets of Langerhans	Insulin
Pituitary	
Anterior Lobe	Growth Hormone
	Gonadotrophic Hormones
	(1) Follicle stimulating hormone
	(2) Luteinising hormone
	(3) Luteotrophic hormone (Lactogenic hormone)
	Thyrotrophic Hormone
	Adrenocorticotrophic Hormone
Posterior Lobe	Oxytocic Factor
	Pressor Factor
Adrenal	
Medulla	Adrenaline
	Noradrenaline
Cortex	Numerous steroid ketones
	e g corticosterone cortisone deoxycortone
Gonads	
Ovaries	α Œstradiol
Corpus Luteum	Progesterone
Testes	Testosterone

The parts of the pancreas known as the islets of Langerhans are responsible for the secretion of the protein insulin which regulates the assimilation of sugars and controls the concentration of glucose in the blood The remainder of the pancreas produces enzymes which when introduced into the alimentary tract break down proteins carbohydrates and fats the islets of Langerhans are interspersed in the glandular tissue which produces these enzymes Much work has been carried out on the chemistry of insulin and the size of the molecule and the nature and number of the amino-acids of which it is composed are now known with a fair degree of certainty although the order in which the several different amino-acids are joined together in insulin is not completely known

The pituitary gland is composed of two distinct parts known as the anterior and posterior lobes each lobe arises from different embryonic tissues In man it is situated in the centre of the head at the base of the brain The whole gland is about the size of an acorn and usually weighs

rather less than one gramme The anterior lobe is the source of a number of hormones which have far reaching effects on the functions of other glands especially the gonads It has been asserted that the anterior lobe controls the activity of the whole endocrine system and may thus be regarded as the master gland Among the active principles arising from the anterior lobe are a growth promoting hormone a thyroid stimulating hormone gonadotrophic hormones and a lactogenic hormone The function of the posterior lobe is less clear but it appears to secrete two hormones which contract the uterus and intestines respectively Little is known of the chemical nature of these substances

The adrenal glands or suprarenals are two in number In man they weigh about four grammes each and are placed above and in front of the kidneys Each gland consists of two parts the medulla and the cortex *The medulla which is the central portion produces the hor*mones adrenaline and noradrenaline which are basic derivatives of catechol Adrenaline was the first hormone to be isolated in pure form and both adrenaline and noradrenaline have been synthesised by several methods Adrenaline which serves to mobilise the resources of the body to meet an emergency causes acceleration of the heart beat retardation of the digestive processes and liberation of the carbohydrate reserve The cortex produces a large number of steroid hormones including corticosterone dehydrocorticosterone deoxycortone cortisone adrenosterone and many others Many of these have been synthesised from sterols or bile acids In addition small quantities of some of the sex hormones are found in the cortex Certain of the hormones of the adrenal cortex are essential to life

The gonads are the glands chiefly concerned with the secretion of the sex hormones In the female they consist of the ovaries in the male the testes The cells of the ovaries and testes are responsible for secreting their hormones into the blood at a very early stage in human development and this results in the development of female or male characteristics During childhood further sexual differentiation is to a large extent arrested to be resumed with greater effect at puberty until full maturity has been reached usually in the early twenties The ovaries of the adult woman are a pair of egg shaped organs situated below the fallopian tubes in the upper segment of the pelvis At approximately monthly intervals from puberty to the menopause an ovum is discharged from its follicle in the ovary This ruptured follicle then becomes filled with luteal cells containing a yellow fatty substance and finally a new small discrete organ known as the corpus luteum projects from the surface of the ovary The ovaries produce the female or follicular hormone known as α –œstradiol which is classified as an œstrogen while the corpus luteum produces a second hormone progesterone Both hormones are steroid in character and have been synthesised from sterols in the laboratory Other closely related substances such as œstrone œstriol and pregnanediol are found in human

female urine especially during pregnancy These are the forms in which the hormones are excreted The testes of the adult man are paired ovoid organs of about the size of a small hen s egg and are contained in an extension of the body cavity known as the scrotum *The hormone of the testes is testosterone a steroid of known constitution* which can be prepared from cholesterol This hormone is termed an androgen and other androgens such as androsterone and dehydro*iso*androsterone which are the metabolic products of testosterone to which they are closely related chemically have been isolated from male urine

The endocrine glands do not in general function in isolation and independently of each other and different glands may affect biological processes in different ways Whereas the thyroid hormone stimulates the oxidation of sugars adrenaline liberates sugar from the liver and insulin controls the assimilation of sugars from the blood The different hormones may therefore either antagonise or reinforce the action of each other Of even greater importance are the more direct influences which the secretions of one gland have on the functions of others and this is best illustrated by the powerful and fundamental effects throughout the whole endocrine system which can be traced to the anterior lobe of the pituitary

CHAPTER I

HISTORY

THE origin of endocrinology can be traced back to the earliest times when man attributed special virtues to the internal organs obtained from slain enemies or animals but modern interest in hormones is often said to date from the sensational experiments of Brown Sequard reported before the Societe de Biologie in Paris in 1889 At the age of seventy two he treated himself for declining vigour with injections of aqueous extracts of the testes of dogs and guinea pigs but the beneficial results which he claimed can be attributed only to over enthusiasm and auto suggestion Many years earlier in 1849 Berthold had carried out experiments on the castration of male chicks which resulted in the development of capons large sexless birds without comb wattles spurs and other accoutrements of avian masculinity further when testes were transplanted under the skin of the capons the masculine characteristics developed Although the analogy between the testes and the ovaries was recognised by early anatomists the changes which result from the removal of the ovaries were discovered much later than those resulting from the removal of the testes and it was not until towards the end of the nineteenth century that it was also found that the transplantation of ovaries into castrated animals resulted in the temporary reversal of the changes brought about by castration The failure of attempts to treat male deficiencies and more particularly to effect rejuvenation by means of the oral administration of dried gland tissue or by means of the parenteral administration of aqueous extracts led naturally to the experiments on the grafting of animal testicles by Voronoff and indirectly to the device of the ligation of the vas deferens by Steinach but both ventures in spite of early claims were doomed to failure as a solution to the many sided problem of senescence Both the grafting of testicular tissue and the tying of the vas deferens were designed to increase the concentration of the male hormone

Adrenals The first insight into the functions of the adrenal glands was provided by Addison who in 1849 showed that their destruction led to the disease which now bears his name This disease which is characterised by vomiting bronzing of the skin and muscular weakness can now be treated successfully by administration of one of the hormones found in the adrenal cortex Active chemical work on the nature of the compounds present in the cortex followed much later and dates from about 1930 Already some twenty eight different steroids have been isolated and identified from this source mainly in the laboratories of Reichstein in Switzerland and of Kendall and Wintersteiner in America Among these is cortisone the successful use of which in the treatment of rheumatic diseases was first reported by

Hench and Kendall in 1949 The first observations on the functions of the adrenal medulla are usually attributed to Oliver and Schafer who in 1894 observed the effects on man of extracts of the adrenal glands which among other reactions cause an increase in blood pressure In 1901 the active principle adrenaline was isolated in pure form and three years later it was the first hormone to yield to synthesis in the laboratory The occurrence of noradrenaline in the medulla was reported in 1949

Thyroid In 1856 Schiff found that dogs and guinea pigs could not survive the removal of their thyroid glands and that the animals could be saved by transplanting the glands at any point under the skin The fact that excessive thyroid secretion might be the cause of exophthalmic goitre was first suggested by Rehm in 1884 These observations not only established the importance of the gland but also led to the successful use of gland extracts in the treatment of thyroid deficiency as revealed in cretinism and myxoedema The isolation of the active principle in pure form was not achieved until 1919 eight years later the synthesis of thyroxine was reported by Harington and Barger

Pancreas The recognition of some connection between sweet urine and the pancreas dates back to the early years of surgery but the cause of diabetes mellitus remained enveloped in mystery until 1889 when von Mehring and Minkowski observed that the removal of the pancreas from dogs resulted in the production of urine very rich in sugars The dogs became weak and finally died after having shown all the symptoms associated with diabetes mellitus In this way the function of the pancreas became associated with diabetes mellitus and this suggestion was confirmed when it was observed that the pancreas of human patients who had died as a result of diabetes mellitus showed signs of damaged cells Many attempts were made to isolate from the pancreas an active principle which could be administered to diabetic patients but success did not come until 1919 when Banting showed that the active principle to which the name insulin was given was sensitive to and destroyed by the trypsin in the pancreatic juice By tying the pancreatic ducts in dogs and allowing a period of time for the degeneration of the existing trypsin Banting and Best were able to prepare for the first time extracts which could relieve diabetes mellitus when administered by subcutaneous injection and it was in 1922 that the first human patient was treated successfully with insulin

Parathyroids In the decade which followed the discovery of insulin attention was directed to the isolation of the active principles of many other glands and Collip and Hanson isolated from the parathyroid glands a potent extract which was shown to have the property of preventing tetanic convulsions and of controlling calcium metabolism

Pituitary At about the same time important developments in connection with the pituitary gland were reported by Evans and Long From the anterior lobe they isolated an extract which when injected subcutaneously into rats caused rapid and abnormal growth and brought about fundamental changes in sex development It was later demonstrated by Smith that normal growth was resumed when crude anterior lobe extracts were administered to dwarfed hypophysectomised rats The relation between the pituitary and sex maturity was confirmed when Aschheim and Zondek showed that a similar active principle the gonadotrophic factor was present in human pregnancy urine an observation which later led to the development of the Aschheim Zondek test for pregnancy A new source of gonadotrophic hormones was discovered by Cole and Hart in 1930 in the blood serum of pregnant mares The exact number of active principles associated with the gonadotrophic factor is uncertain but at least two different hormones are usually recognised namely the follicle stimulating hormone and the luteinising hormone A relation between the pituitary and thyroid glands was first suggested by Rogowitsch in 1888 and confirmed by later workers Evidence was also obtained of the existence of an adrenocorticotrophic substance and of a lactogenic or lactation hormone (prolactin) The anterior lobe of the pituitary thus affords a considerable variety of factors all of which appear to be proteins The complete separation of the different factors from each other is a problem beset with great practical difficulties which are further complicated by a lack of precise knowledge of the extent to which activity is due to a specific hormone or to a combination of hormones The relation between the posterior lobe of the pituitary and diabetes insipidus was recognised by Vassale and Sacchi in 1893 and in 1906 Dale observed that an extract of the posterior lobe could cause contraction of the muscle of the uterus

Gonads The remarkable advances which have been made in our knowledge of the sex hormones which with the hormones of the adrenal cortex may be classified as the steroid hormones began in 1923 when Allen and Doisy demonstrated that the follicular fluid obtained from animals ovaries contained an active œstrogenic hormone which six years later was isolated in pure crystalline form from human pregnancy urine Shortly afterwards a second hormone œstriol was isolated by Marrian to be followed later by the isolation in Doisy s laboratory of α œstradiol from the follicular fluid of sow ovaries α Œstradiol had previously been prepared in the laboratory by Schwenk and Hildebrandt by the reduction of œstrone As early as 1903 Fraenkel had observed that the removal of the corpora lutea from rabbits in early pregnancy was followed by abortion but it was not until 1928 that evidence was obtained by Weichert of the existence of a specific corpus luteum hormone This hormone progesterone was isolated in pure

form by Butenandt in 1934 and was synthesised in the same year In *the male hormone field Butenandt reported in 1931* the isolation of androsterone from male urine and later work by Koch and McGee in Chicago and by Laqueur in Amsterdam led to the isolation of the male hormone testosterone Soon after its discovery androsterone was prepared in the laboratory from cholesterol by both Ruzicka and Butenandt and this achievement was shortly afterwards followed by the preparation of testosterone itself

Chemical Constitution It will be seen that as with the vitamins the hormones do not belong to any one specific class of chemical compound Some like adrenaline and thyroxine are relatively simple *derivatives of benzene others including all the sex hormones as well* as the hormones of the adrenal cortex belong to the steroid group yet others such as insulin and the hormones of the pituitary are typical *proteins Adrenaline and thyroxine being the hormones of simplest* constitution were the first to be synthesised but the more complex sex hormones and hormones of the adrenal cortex have also been synthesised by many different methods which make use of either sterols bile acids or more recently sapogenins as starting materials These are only partial syntheses in that they start with a preformed steroid nucleus but in one or two cases the total synthesis of a steroid hormone has been achieved in spite of the complexity of the stereochemical difficulties which have to be overcome The total synthesis of all the steroid hormones may now be said to be within sight of achievement On the other hand exact knowledge of the constitution of the protein hormones is still lacking and their synthesis remains a distant objective *In some instances notably with the œstrogenic hormones* considerable success has been obtained in the preparation of artificial compounds which appear to possess all the properties of the natural hormones and many such preparations have been introduced into medical practice

Clinical Applications It is obvious that there is now an extensive fund of knowledge anatomical physiological and chemical on the nature and functions of the endocrine glands and their hormones and the study of endocrinology is recognised as an essential part of the medical curriculum Its practical application in the treatment of disease has many notable achievements to its credit but not infrequently difficulties have arisen in applying to human patients the results obtained with experimental animals Such difficulties however are not confined to this field alone and are frequently encountered in chemotherapy as well as in other branches of medical research Perhaps the most successful applications in clinical practice are to be found in the uses of insulin thyroxine and adrenaline The use of certain sex hormone preparations and the treatment of Addison s disease with deoxycortone acetate have also been conspicuously successful

CHAPTER II

PHYSIOLOGY

WHEN considering the physiology of the hormones of the endocrine glands it is important to take into account the interrelation that exists between the different hormones and because of the dependence of one gland upon another a logical approach to the subject is difficult For proper physical and mental development all the endocrine glands in the body must be functioning and a delicate balance of the amount of hormone secreted by each gland must be maintained

Zondek[1] states that hormones mutually promote or inhibit one another inasmuch as each individual hormone may stimulate or inhibit the activity of other hormonal glands In other words an under or overactive gland leads to changes in other glands and the effects of dysfunction must be considered in relation to the endocrine system as a whole

THYROID

The thyroid consists of two lateral lobes together weighing about twenty five grammes in a human adult which are joined together by the isthmus a narrow piece of similar tissue It is situated in the neck on the upper portion of the trachea and produces the hormone thyroxine

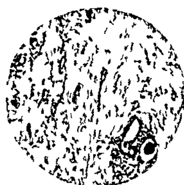


FIG ?

POSITION OF THYROID

The two lobes of the human thy o d lie in the neck on e ther side of the larynx and upper t achea The isthmus which connects them l es in front of the upper four rings of the trachea

Hypothyroidism The thyroid regulates the production of energy and controls physical and mental development The result of an under active thyroid gland is myxœdema or hypothyroidism congenital absence of the gland causes cretinism In adults thyroid insufficiency is indicated by well marked and typical symptoms all the metabolic processes of the body are slowed down the skin becomes dry coarse and puffy the hair becomes brittle and coarse and there is general lethargy In children there is a retarded state of development both physical and mental the heart rate is slow and the children are generally apathetic The administration of thyroid by mouth results in *a dramatic improvement in myxœdema but in cretins thyroid therapy* to be effective must be instituted early in life



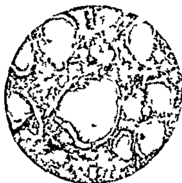




FIG 3

SECTIONS OF THYROID

A Myxœdematous tissue showing reduced vesicles scattered in fibrous tissue (× 400) *B* *Normal tissue* showing vesicles filled with gelatinous colloid embedded in connective tissue (× 400) *C* Toxic goitre tissue showing increased vascularity with *many vesicles containing little colloid* (× 400)

Hyperthyroidism An overactive thyroid leads to hyperthyroidism or primary thyrotoxicosis (toxic goitre) Other names given to diseases associated with an overactive thyroid gland are Graves, Parry s or Basedow s disease and exophthalmic goitre *Authorities differ* as to the best nomenclature Exophthalmos bulging of the eyes is not always present and the term most frequently used is Graves disease

Sir Robert Graves first described the symptoms in 1825 before the effects of the administration of thyroid had been observed The symptoms of this condition are an increase in the heart rate and a general state of anxiety and restlessness sometimes especially in severe cases accompanied by exophthalmos

Microscopical Characters The thyroid gland as shown by a microscopical examination of a section consists of a large number of vesicles embedded in connective tissue and filled with a viscous gelatinous material generally referred to as colloid which passes into the blood stream either directly or through the lymphatic system The amount of colloid excreted is regulated by the thyrotrophic hormone of the anterior lobe of the pituitary Microscopical examination of the gland in myxœdema shows delicate fibrous tissue in which the remains of vesicles are scattered In hyperthyroidism the thyroid shows increased vascularity with many vesicles containing little or no colloid

Thyroid and Metamorphosis Minute amounts of thyroid greatly accelerate the change of tadpoles into frogs The accelerated metamorphosis occurs when the tadpoles are kept in water containing thyroid or thyroxine and is most useful as a test in the biological investigation of compounds for thyroid activity Substances other than

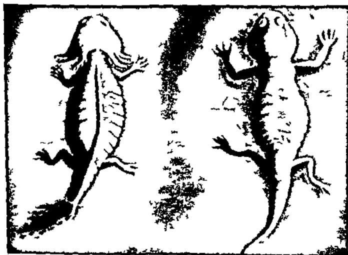


FIG 4

INDUCED METAMORPHOSIS IN AXOLOTL

The Mexican salamand r (*Ambystoma ti num*) or axolotl usually undergoes no metamorphos s but breeds n the g lled state (*l ft*) when fed on thyroid it develops a terrestrial form (*ght*)



Fig 5

South African Clawed Toad (*Xenopus laevis*)

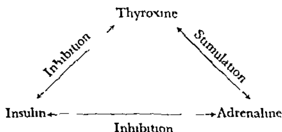
The development from tadpole to toad is normally complete in 10 to 12 weeks but may be hastened by substances containing thyroxine such as thyroid and iodinated casein The photographs show tadpole (*top left*) intermediate stage (*top right*) male toad (*bottom left*) and female toad (*bottom right*) The female toad is also used in the xenopus (Hogben's) pregnancy test

thyroxine however are able to produce in mammals a response similar to that given by thyroid feeding and the effect on tadpoles does not provide a satisfactory method for the assay of thyroid preparations

Frogs formed by feeding with thyroid are however dwarfs and soon die since thyroid accelerates their metamorphosis but inhibits their growth It has been shown that the thyroids from various forms of goitre differ greatly in their specific influence on the tadpole [2]

Another example of the action of the thyroid hormone is seen when the Mexican salamander or axolotl is fed on thyroid In the normal state the axolotl does not usually undergo metamorphosis but the administration of thyroid or thyroxine induces a metamorphosis resulting in a land animal Iodine alone is incapable of inducing such a change since the axolotl has an undeveloped thyroid

Thyroid and Other Endocrine Glands The following diagram indicates a possible relation between the thyroid and other endocrine glands —



The thyroid hormone for example is thought to be necessary for the normal action of adrenaline and it is likely that an excess of thyroxine either stimulates the production of extra adrenaline or sensitises the tissues to its action Thyroxine and insulin are in certain respects antagonistic and a person with an underactive thyroid has an abnormally high sugar tolerance diabetes mellitus is frequently associated with increased activity of the thyroid Thyroid enlargement may occur at puberty at the climacteric and also during pregnancy and menstruation This may be due to an increased secretion of the ovarian hormones or to a deficiency of iodine brought about by increased metabolism at these times Thyroidectomy frequently causes degeneration of the gonads in males and females

Basal Metabolic Rate In the diagnosis of thyroid disorders a determination of the heat production capacity or basal metabolic rate (B M R) is most valuable The standard is the heat production of a person in a fasting condition at rest and the basal metabolic rate is expressed as a percentage above or below the normal after taking age

height and weight into consideration When the heat produced is greater than normal the basal metabolic rate is expressed as plus and when less than normal minus In mild hyperthyroidism patients have a basal metabolic rate of +10 to +30 and in severe hyperthyroidism +30 to +60 Other conditions such as fever can elevate the basal *metabolic rate appreciably but they can usually be easily differentiated* from a raised basal metabolic rate due to hyperthyroidism

In experimental animals the basal metabolic rate may be found by measuring the heat lost in a calorimeter but in man it is determined indirectly by finding the oxygen consumption over a period in a Benedict Roth apparatus This consists of a tank filled with oxygen suspended in water and connected by means of tubing to the patient s mouth The patient inhales oxygen directly from the tank and exhales back to the tank through a soda lime tower where the carbon dioxide formed is removed The decrease in volume of oxygen in the tank is thus a direct measure of oxygen consumed Before the estimation the patient should fast for twelve hours rest for half an hour and be unexcited[3]

The basal metabolic rate yields valuable information in conditions caused by disturbances of the thyroid and pituitary glands In exophthalmic goitre it is increased by an amount varying proportionately with the severity of the condition It has also been shown that the basal *metabolic rate is lowered for fourteen days in patients with hyper* thyroidism by the administration of Lugol s solution (Aqueous Solution of Iodine B P) and that it then rises again In hypothyroidism the basal metabolic rate is −20 to −40

Radioactive Iodine As a means of studying the absorption and elimination of drugs in the body radioactive elements are proving to be *most useful particularly when the estimation of minute amounts by* chemical means is impossible Comparatively little is known of the fate of thyroxine and iodine in the body but it has been shown by administering radioactive iodine that more than 50 per cent of the amount administered appears in the thyroid gland In hypothyroidism the thyroid gland utilises little or no iodine in simple goitre more iodine is taken up and in toxic goitre or hyperthyroidism although more iodine *is absorbed it is not retained* This fact may give a clue to the reason why iodine causes a temporary remission of symptoms in toxic goitre Radioactive iodine has also been used therapeutically for the selective destruction of the active tissue of the thyroid gland by internal radiation when it acts in a manner similar to that of X rays[4] *It is claimed* that a cure rate of 80 per cent can be obtained in patients with hyperthyroidism by treatment with radioactive iodine and it may be that when more precise details have been worked out this method will replace the use of drugs and the surgical removal of a portion of the thyroid gland

Iodinated Proteins Many attempts have been made to prepare physiologically active iodinated proteins which can be used instead of thyroid Rivers and Randall[5] prepared iodinated ox plasma ardein (a mixture of proteins from ground nuts) and ca ein Iodinated casein proved to be the most satisfactory and it was possible by administering it to cows to raise their basal metabolic rate and stimulate the production of an increased yield of milk This use is still under experimental study and cannot be generally recommended until more detailed information is available regarding possible undesirable effects which may follow administration over a long period The activity of these compounds has been shown to be due to the presence of thyroxine and this adds further evidence in proving that thyroxine is the hormone of the thyroid gland[6, 7]

Antithyroid Substances In 1928 it was shown by Chesney and his co workers[8] that ingestion of plants belonging to the genus *Brassica* (family Cruciferæ) especially cabbages produced thyroid enlargement with associated underactivity in rabbits and in 1941 MacKenzie MacKenzie and McCollum[9] while working on the effects of sulpha guanidine in rats discovered that it gave rise to hypothyroidism Later MacKenzie and MacKenzie[10] and Astwood *et al*[11] showed that other sulphonamides also caused hyperplasia of the thyroid gland in rats and many compounds were examined with a view to finding one having an antithyroid action

Astwood[12] showed that thiourea and thiouracil were capable of reducing the ecretion of the thyroid gland and subsequent studies have led to the belief that they act by preventing iodine from combining with tyrosine and consequently the synthesis of thyroxine Subsequently methylthiouracil was shown to exert a more rapid therapeutic action in smaller doses than thiouracil[13, 14] while Astwood *et al*[15] Anderson *et al*[16] and Pavin and Goodchild[17] have found that of over three hundred drugs tested in the rat propylthiouracil has the greatest anti thyroid activity As a result of clinical work with propylthiouracil Astwood and Vander Laan[18] have reported a satisfactory control with no untoward reactions in fifty two patients The administration of thiouracil and its derivatives requires to be carefully controlled ince they are liable to give rise to toxic symptoms agranulocytosis the most dangerou of these mostly occurs from overdosage They should not be used in the treatment of simple goitre when the enlargement of the thyroid is due to underactivity of the gland nor should they be used in the treatment of large toxic goitres which are causing pressure symptoms

Parathyroid

The parathyroid glands are the smallest members of the endocrine system In man they consist of yellowish red ovoid bodies 3 to 15 millimetres in length 2 to 4 millimetres in width and 1 to 2 millimetres

in thickness The average combined weight of the glands is about 0 5 gramme The number and position of the parathyroid glands is extremely variable usually four are situated on the under surface of the thyroid but as many as twelve have been reported

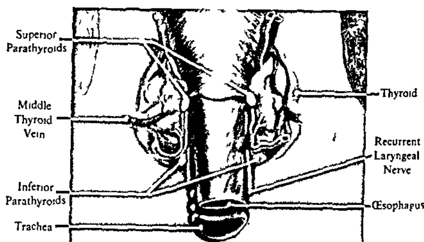


Fig 6
Position of Parathyroids
The four small glands lie on either side of the thyroid with which they have no vascular connection

The name parathyroid is somewhat misleading since these glands have no vascular connection with the thyroid Although they may influence and be influenced by other glands the existence of a para thyrotrophic hormone in the anterior lobe of the pituitary has not been proved and other glandular interrelations are not clear

Effects of Parathyroidectomy The accidental removal of the parathyroids in an operation on the thyroid proved that they were essential to life and experimental work on animals shows that they are associated with calcium and phosphorus metabolism The complete removal of the parathyroid glands is followed by muscular tremors cramps and convulsions ultimately leading to the death of the animal The symptoms are associated with a fall in the calcium content of the blood serum and a retention of acid soluble phosphates Parathyroidec tomised animals can however be kept alive by feeding on a high cal cium and low phosphorus diet

Hypoparathyroidism In man, under secretion of the para thyroid hormone or hypoparathyroidism is associated with tetany the chief symptoms of which are increased nervous excitability and

painful muscular spasms Ectodermal lesions such as loss of hair brittle nails and cataract may occur in patients suffering from long standing tetany Tetany due to hypoparathyroidism is a somewhat rare occurrence it most frequently arises from injury or removal of parathyroid tissue Surgical removal may occur accidentally during thyroidectomy on account of the proximity of the parathyroid and thyroid glands Tetany is also associated with rickets and sometimes with pregnancy and lactation owing to the increased calcium requirements at these times

Hyperparathyroidism Over secretion of parathyroid hormone which may follow a tumour of the gland is characterised by osseous decalcification resulting in softening rarefaction and deformity of the bones There is pain in the bones and other tissues and renal calculi are commonly pre ent In the absence of surgical treatment the progressive deformity and weakness may lead to death

Calcium and Phosphorus Metabolism Since the parathyroid hormone is so intimately concerned with calcium and phosphorus metabolism it is important to consider the function of these elements in the body

The absorption of calcium and phosphorus from the bowel depends primarily on the calciferol (vitamin D) content of the food the calcium phosphorus ratio and the hydrogen ion concentration of the bowel contents Increased acidity in the bowel favours absorption and conversely increased alkalinity gives rise to insoluble calcium salts which are not absorbed If the phosphorus intake is excessive insoluble calcium phosphate is formed and calcium loss occurs The blood serum normally contains about 10 milligrams of calcium and 3 to 5 milligrams of inorganic phosphate per 100 millilitres Should the calcium content fall to 8 to 6 milligrams per 100 millilitres with a proportionate increase in phosphorus then tetany results The skeleton which contains over 99 per cent of the body s calcium acts as a storehouse for calcium and phosphorus from which these elements can be mobilised as required for the blood serum Its mode of action is in some ways comparable to that of the liver which stores carbohydrate as glycogen and releases it as glucose when required

The rate of deposition of calcium in the bones depends upon the calcium ingestion and on the amount of parathyroid hormone present in the blood The parathyroid hormone mobilises calcium from the bones and maintains the correct level of phosphorus in the body fluids and cells

Albright and Reifenstein[19] believe that the primary action of the hormone is on phosphorus metabolism and the changes which occur in the calcium content of the blood serum are secondary to those of phosphorus metabolism

Neufeld and Collip [9] consider that the parathyroid hormone has no direct effect on the bone its primary action being on the excretion of phosphates by the kidneys Albright [1] discussing drugs which influence calcium and phosphorus metabolism points out that vitamin D favours their intestinal absorption while the parathyroid hormone regulates their concentration in the blood serum it does not affect the absorption of calcium and phosphorus in the bowel A phosphatase enzyme is also concerned in bone formation It is believed to be a product of proliferating cartilage cells Alkaline phosphatase has a maximum activity between *p*H 9 and 10 while an acid phosphatase which has been isolated from prostatic epithelium has a maximum activity at about *p*H 4 8 Serum alkaline phosphatase is at a minimum in normal healthy adults and increases whenever bone is being formed or rapidly destroyed in such conditions as hyperparathyroidism Magnesium greatly increases the activity of the enzyme whereas calcium ions are mildly inhibitory

Parathyroid Hormone and Tetany The parathyroid hormone raises the calcium content of the blood serum and *will* thus relieve tetany following parathyroidectomy Its use in man for this purpose however has the disadvantage that it takes about four hours to act and injections may cease to be effective after two or more weeks *owing to the supposed development of antihormones*

Robertson[2] discussing the various methods used in the treatment of parathyroid tetany refers to the dangers of overdosage loss of effectiveness or decalcification of the bones if parathyroid preparations are *used over long periods He found that thyroid or thyroxine temporarily* controlled tetany and concludes that calcium salts either by injection or orally still remain the chief therapeutic measure Shelling [3] found that following an injection of 10 millilitres of a 10 per cent solution of calcium gluconate there was a rapid increase in the percentage of serum calcium (up to 100 per cent increase at the end of injection) The serum calcium fell however to the previous level in two to four hours

Dihydrotachysterol and Calciferol In recent years interest has been aroused in a substance known as dihydrotachysterol or AT 10 (anti tetany 10) a by product formed during irradiation of ergosterol in the manufacture of calciferol This compound was first isolated and used clinically by Holtz in 1927 who reviewed his results *in 1941*[24] *It* is administered by mouth in solution in oil and will abolish tetany in two to three days slowly raising the serum calcium to normal levels Dihydrotachysterol is considered to have an effect intermediate *between that of calciferol and the parathyroid hormone* In its method of action however it appears to resemble the parathyroid hormone more than calciferol and it has no appreciable antirachitic effect

Sevringhaus and St John [5] found the oral administration of large doses of calciferol combined with calcium salts effective in treating tetany and consider that calciferol is apparently as safe as dihydro tachysterol A dose of 100 000 units of calciferol daily for two weeks raises the serum calcium level but is without effect on the inorganic phosphorus

Brack [6] has made the interesting observation that in a patient who had developed tetany following thyroidectomy and had received treatment with dihydrotachysterol and calcium intravenously the tetany could be controlled with a very much smaller dose of dihydrotachysterol without calcium if a histamine antagonist were administered at the same time no explanation of the mode of action is suggested

PANCREAS

The pancreas or sweetbread is a relatively large gland analogous in structure to the salivary glands but softer and less compactly arranged It is situated in the upper abdomen and weighs about 75 grammes

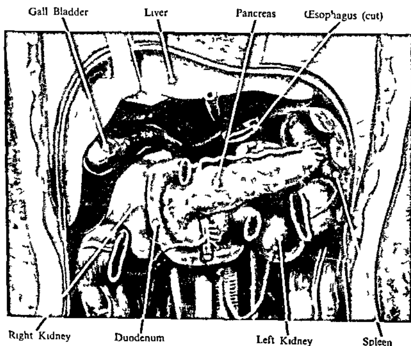


FIG 7

POSITION OF PANCREAS

This large gland (approximately 7 in by 1½ in) lies in the upper abdomen behind the stomach and extends somewhat horizontally from the duodenum to the spleen

The pancreas is a compound organ consisting of cells which produce enzymes which aid digestion interspersed throughout the gland are *clusters of cells called islets of Langerhans* which are responsible for the secretion of the hormone insulin These small islets form a thirtieth to a hundredth of the mass of the pancreas and it is generally considered that the acini which secrete enzymes and the islet tissue are distinct one producing an external secretion which is conveyed through the pancreatic ducts into the duodenum while the other produces insulin which is carried directly into the blood stream by small capillaries in the centre of the islet

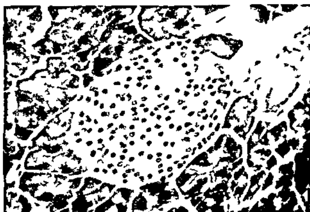


FIG 8

SECTION OF MAMMALIAN PANCREAS

Showing an islet of Langerhans The islet cells are responsible for the secretion of insulin which passes directly into the blood stream

Two types of cells are present in the islets alpha cells whose granules are insoluble in alcohol and beta cells whose granules are soluble in alcohol *It has been shown that the beta cells are those which secrete* insulin

Carbohydrate Metabolism The chief sources of bodily energy are sugars starches and glycogen Carbohydrates are usually ingested as starches and disaccharides and these are converted into mono saccharides in the body and then into glycogen which is stored in the liver and muscles *The liver can also convert amino acids into glycogen* and it acts as a storehouse from which the body obtains glucose as required by the action of the enzyme glycogenase in the liver About 500 grammes of glycogen are stored in the body 100 grammes being stored in the liver and most of the remainder in the skeletal muscles Insulin is essential for the correct metabolism of carbohydrates and

fats and the most characteristic effect of an injection of insulin is to reduce the amount of sugar in the blood

Action of Insulin Insulin has three main actions it aids the combustion of glucose by the tissues it favours the formation of glycogen in the liver and muscle and it inhibits glucose formation from amino acids in the liver When there is a deficiency of insulin sugar appears in the urine and diabetes mellitus results This is however only an indication of a complicated derangement in metabolism which is taking place glycogen reserves in the liver are rapidly depleted and when they are exhausted body proteins are utilised In the later stages fat is drawn upon resulting in fat derivatives or ketone bodies such as acetone making their appearance in the blood and urine

If diabetes mellitus is untreated diabetic coma may result It may also occur in diabetic patients following infections such as influenza or pneumonia owing to an increased insulin requirement in these conditions Coma is due to gross impairment of the fat metabolism resulting in the formation of ketones which exert toxic action particularly upon the central nervous system Ketone bodies including acetoacetic acid β-hydroxybutyric acid and acetone appear in the urine and the characteristic sweet smell of acetone can usually be detected in the patient s breath

Blood Sugar Levels There is no reliable method for the determination of the amount of insulin in small quantities of blood and the diagnosis of diabetes mellitus is usually made by estimating the amount of glucose in the urine and in the blood The normal fasting blood sugar level is about 100 milligrams per 100 millilitres it rises to 130 to 170 milligrams per 100 millilitres after a meal returning to normal in about two to three hours Thus although 200 grammes of glucose may be absorbed after a meal the rise in blood sugar represents an increase in circulating glucose of only about three grammes This constancy is lacking in severe untreated diabetes where the fasting level may be 130 milligrams per 100 millilitres rising to 200 to 500 milligrams per 100 millilitres and remaining above 180 milligrams for the greater part of the day Below a certain blood sugar level known as the leak point or renal threshold no sugar is excreted by the kidneys When the blood glucose level exceeds this threshold glucose passes through the kidneys and into the urine In some people the renal threshold is below 180 milligrams per 100 millilitres and sugar appears in the urine even though the blood concentration is not abnormally high This condition is known as renal glycosuria or diabetes innocens and requires no treatment Lawrence and McCance[27] point out that renal glycosuria may be the cause of a diagnostic error if the possibility of a low renal threshold is not considered in patients presenting glycosuria without typical symptoms of diabetes mellitus

Glucose Tolerance Test To confirm the diagnosis of diabetes mellitus blood sugar examinations are frequently carried out before and after the administration of glucose and this test is called a glucose tolerance test The patient is allowed an unrestricted carbohydrate diet for several days and a blood sugar determination is made in the morning after the patient has fasted since the evening meal of the previous day The patient then drinks a glass of water containing 50 grammes of glucose and further blood sugar determinations are made at half hourly intervals for two hours Samples of urine are also collected and tested for sugar and ketone bodies The blood sugar values are plotted and Fig 9 illustrates the difference between the blood sugar concentration of a normal person and of a person with diabetes mellitus

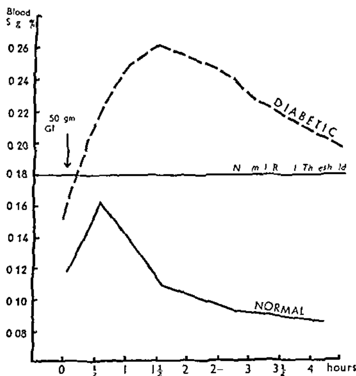


Fig 9
Glucose Tolerance Test
The graphs indicate the changes in the blood sugar levels following the administration of 50 grammes of glucose to a normal and a diabetic subject

Insulin Preparations The type of preparation which has been longest in use consists of a simple solution from which however the insulin is so rapidly absorbed by the body that two or more injections are needed each day to maintain the action of the insulin Attempts

have therefore been made to modify the action of insulin in order to give a slow prolonged effect The first real advance was achieved in 1936 and 1937 by Hagedorn [8, 29] who found that protamine obtained from the sperm of salmon trout when mixed with insulin and a suitable buffer to give a final reaction of pH 6 2 produced a precipitate of protamine insulin which had a low solubility in the tissue fluids and was only slowly absorbed Scott and Fisher[30, 31, 3] found that the addition of a very small percentage of zinc to the insulin and protamine prolonged their action The zinc also stabilised the protamine insulin suspension so that it was possible for it to be issued in one vial ready for injection This preparation known as protamine zinc insulin has supersed d protamine insulin

In 1937 Bavin and Broom[33, 34] investigated the effect of different substances on the absorption of insulin and showed that whereas small concentrations of metal produced a prolongation of hypoglycæmia increasing the amount of metal present eventually resulted in a complete inhibition of the normal insulin response They also confirmed the observations of Bischoff[35] and Gray[36] that tannic acid prolongs the hypoglycæmic action of insulin and that the addition of zinc to the tannic acid insulin complex further prolongs insulin hypoglycæmia

Reiner *et al* [37] have prepared a combination of insulin with globin a protein obtained from the red blood cells of the ox by removal of the chromogen fraction with the addition of a trace of zinc chloride to the mixture They found that globin zinc insulin had an action which was intermediate in intensity and duration of effect between soluble insulin and protamine zinc insulin

The relative action of the three insulins is shown in Fig 10

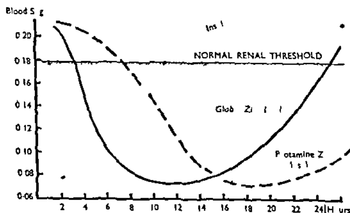


FIG 10

I SULIN ABSORPTION RATES

The rapid absorpt on of insul n the more prolon ed absorpt on of globin z nc insul n an l the low dur t on of ff ct of prota mine nc insulin are h wn by the th ee curves on th graph

Biochemical Examinations In diabetes mellitus biochemical examination of the blood for sugar and of the urine for sugar and acetone bodies or ketones is essential in diagnosis and for ascertaining the amount of insulin required for treatment The determination of glucose in the blood and urine both depend upon the fact that glucose in alkaline solution is a powerful reducing agent

Sugar in Urine—Qualitative Tests (1) Benedict s Test Urine examinations for sugar can be carried out easily by a diabetic patient using Benedict s qualitative reagent which is prepared as follows —

Sodium Carbonate	200 0 g
(or Exsiccated Sodium Carbonate	90 0 g)
Sodium Citrate	173 0 g
Copper Sulphate	17 3 g
Distilled Water	to 1000 0 ml

Dissolve the sodium carbonate or the exsiccated sodium carbonate in 600 ml of water with the aid of gentle heat add the sodium citrate cool and with constant stirring slowly add a cold solution of the copper sulphate in about 100 ml of distilled water add sufficient distilled water to produce the required volume

To carry out the test 5 ml of Benedict s qualitative solution is added to 8 drops of urine in a test tube the mixture boiled for two minutes and allowed to cool If glucose is present the reagent changes from clear blue to green yellow or brick red On standing reduced copper settles to the bottom of the tube as a reddish yellow deposit 0 1 per cent of sugar gives a very slight reduction and the fluid turns deep blue on standing 1 per cent of sugar produces a heavy precipitate but there is still some blue colour in the supernatant liquid

(2) Fermentation Test To confirm that the reducing substance present in the urine is glucose a fermentation test may be carried out A trace of tartaric acid is added to a sample of the urine the specific gravity is determined and some baker s yeast is added The mixture is allowed to stand in a warm room for twenty four hours and the specific gravity again determined If glucose is present fermentation will have taken place and there will be a fall in the specific gravity An alternative method is to ferment the urine in an inverted test tube or a Doremus ureometer and collect the carbon dioxide produced by the fermentation of the glucose It is advisable to put on control tubes one with yeast and glucose to make sure that the yeast is active and one with yeast and normal urine to make sure that the yeast contains no glucose

Sugar in Urine—Quantitative Tests More precise quantitative methods for determining the amount of glucose in the urine are only occasionally required and are described fully in standard textbooks In Benedict s quantitative test the volume of urine which is required to dispel the blue colour from 25 ml of the reagent is determined the urine being diluted if necessary so that the volume of diluted urine required is about 10 ml The colour of 25 ml of Benedict s quantitative

reagent is dispelled by 0 05 g of glucose and from the figures obtained the percentage of glucose in the urine may be calculated

Ketone Bodies in Urine Two tests are available for detecting acetone bodies in urine Rothera s nitroprusside test which is extremely sensitive and shows the presence of both acetone and acetoacetic acid and Gerhardt s ferric chloride test which if positive indicates dangerous acidosis requiring immediate treatment with insulin

(1) Rothera s Nitroprusside Test An excess of ammonium sulphate is added to a test tube half full of urine followed by a few drops of a freshly prepared dilute solution of sodium nitroprusside and 2 ml of strong solution of ammonia The mixture is shaken and allowed to stand The presence of acetoacetic acid or acetone is indicated by a light mauve to deep purple colour

(2) Gerhardt s Ferric Chloride Test A 10 per cent solution of ferric chloride is added to a test tube half full of urine drop by drop until the precipitate of ferric phosphate has reached its full intensity and has wholly or partly redissolved the solution is then filtered A few more drops of the ferric chloride solution are added to the filtrate and the presence of ketones is shown by the development of a claret red colour A similar colour is produced by salicylic acid which may be present in the urine of patients who have taken aspirin or salicylates The colour due to salicylic acid is not destroyed by boiling whereas acetoacetic acid is changed into acetone by boiling and the colour therefore is destroyed

Sugar in Blood For determining the amount of sugar in the blood the Folin and Wu and the Hagedorn and Jensen methods are employed

Before the blood sugar determination is carried out proteins must be removed In Folin and Wu s colorimetric method a 10 per cent solution of sodium tungstate and dilute sulphuric acid is added to the diluted blood The tungstic acid formed precipitates the proteins and allows a clear filtrate to be obtained The filtrate is boiled with an alkaline copper solution and the glucose reduces an equivalent amount of copper to the cuprous state Cuprous salts produce an intense blue colour with a mixture of phosphoric and molybdic acids which at the same time destroys the blue colour due to unchanged cupric salts By comparing the blue colour produced with that obtained under similar conditions with standard glucose solutions the amount of glucose in the blood can be determined

In Hagedorn and Jensen s titration method a protein free filtrate is obtained by adding a measured quantity of blood to a mixture of zinc sulphate and sodium hydroxide in a test tube which is then heated in a boiling water bath for three minutes and filtered The glucose in the filtrate is determined by boiling with potassium ferricyanide which is reduced by the glucose to potassium ferrocyanide Potassium ferricyanide liberates iodine quantitatively from potassium iodide in acid

solution The iodine liberated is determined by titration with sodium thiosulphate solution Two determinations are made one with ferri cyanide which has been boiled with a portion of the blood filtrate and the other with ferricyanide which has been boiled with an equivalent amount of water The difference in the two readings is proportional to the amount of glucose in the volume of blood filtrate taken

Pancreas and Other Endocrine Glands Much of our knowledge of the physiology of the pancreas has been derived from experimental studies of diabetes mellitus in animals Banting and Best isolated insulin from the pancreas glands of animals and proved its value in the treatment of diabetes mellitus

In 1924 it was shown that if the anterior lobe of the pituitary gland were removed the animal became more sensitive to insulin and much interest was taken in dogs whose pancreas and pituitary glands had been removed These experimental dogs the so called Houssay animals survived longer than depancreatised animals probably be cause the removal of the anterior lobe eliminated a hormone acting antagonistically to insulin Later it was found[38] that when extracts of the anterior lobe were injected into dogs or rabbits hyperglycæmia and glycosuria developed suggesting that hyperactivity of the anterior lobe might be the cause of diabetes mellitus Clinically Atkinson[39] has shown that about 30 per cent of patients with acromegaly a disease due to over secretion of the hormones of the anterior lobe had glyco suria or diabetes mellitus thus confirming the relation between the anterior lobe and the pancreas The interrelation between the anterior lobe and the pancreas is further complicated by the fact that according to Marks and Young[40] an insulin increasing or pan creatrophic substance is also present in the anterior lobe of the pituitary gland

The adrenal gland has a diabetogenic effect and Ingle[41, 42] showed that marked hyperglycæmia and glycosuria occurred in normal rats fed with a diet rich in carbohydrate when they were injected with large doses of cortisone

De Finis and Houssay[43] demonstrated that a permanent diabetic condition is produced after a few weeks of thyroid feeding in animals whose pancreatic tissue has been partly removed The blood sugar rises and glycosuria, ketonuria and other signs of diabetes mellitus appear Discontinuance of thyroid treatment is followed by a rapid return to a normal blood sugar level and recovery of the beta cells in the islets of Langerhans which have not been completely removed Houssay[44] states that thyroid treatment can produce diabetes mellitus only in animals with a pancreas reduced by surgical removal or already damaged It cannot do so in animals with an intact and healthy pancreas The thyroid gland increases the rate of carbohydrate meta bolism and decreases the sensitivity to insulin John[45] found that

diabetes mellitus is twice as frequent in patients with hyperthyroidism as in the general population

Ingle[46] also showed that the artificial oestrogen stilboestrol produced glycosuria even after removal of the anterior lobe of the pituitary gland and the adrenals

Alloxan Diabetes In 1937 Jacobs[47] showed that the intravenous administration of alloxan an oxidation product of uric acid produced severe hypoglycæmia and convulsions in rabbits and in 1943 Dunn *et al*[48] showed that the islets of Langerhans in rabbits which have been injected with alloxan are partly or completely destroyed

It is now known that the injection of alloxan is followed by a rise in blood sugar which subsequently falls to convulsant levels if carbohydrates are withheld The effect can be mitigated by appropriate feeding but twenty four hours after injection the blood sugar level is still low Further feeding causes the blood sugar level to rise steeply and from this point on the animal is typically diabetic

TABLE II

EFFECT OF ALLOXAN ON BLOOD SUGAR

Time in Hours	0	2	3	5	7	8	9	24	2	26	31	48
Blood sugar mg /100 ml	100	310	360	115	105		330	90		400	550	600
Treatment	Alloxan 150 mg /kg					Fed			Fed			

Ridout *et al*[49] showed that alloxan produces temporary hypoglycæmia because it causes atrophy of the islet cells and allows their insulin content to be leached out into the blood stream

Dunn *et al*[48] discussed the possibility that alloxan may be formed in the body under physiological conditions and suggests that it may be a possible cause of an initial disturbance of the hormone production of the pancreas gland resulting in diabetes mellitus This work with alloxan is of importance for it shows that a chemical substance can destroy the islets of Langerhans producing all the signs and symptoms of diabetes mellitus in animals but so far little evidence has been obtained that alloxan gives rise to diabetes mellitus in man

Phloridzin Diabetes Phloridzin a substance obtained from the root bark of the apple tree has been used in experimental animals to study carbohydrate metabolism When it is injected the kidney is no longer able to retain glucose large amounts of which appear in the urine as in diabetes mellitus

Lipocaic In 1924 Allan et al[50] showed that although the glycosuria in depancreatised dogs could be controlled by injections of insulin the animals failed to survive for more than a few months and usually died with an extensive fatty infiltration and degeneration of the liver If however the dogs received sufficient raw pancreas in the diet insulin kept them alive indefinitely This curious phenomenon has been investigated by many workers

Dragstedt et al[51] produced an extract of pancreas containing a hormone termed lipocaic which is found in the pancreatic juice and is considered to be essential for the prevention of fatty degeneration of the liver Best[5] has pointed out that lecithin and choline can produce the same effect as lipocaic Successful results have been achieved by the oral administration of lipocaic to a patient with an enlarged liver due to fatty infiltration whereas diabetic children with hepatomegaly have shown marked diminution in the size of the liver after the administration of lipocaic Dragstedt[53] suggests that lipocaic may find a place in the control of liver enlargement and possibly arteriosclerosis but this work has not been confirmed and lipocaic is at present more of theoretical than of practical importance

Pituitary

The pituitary gland or hypophysis is a small but most important gland which is attached to the under surface of the brain It is composed essentially of two main lobes the anterior or glandular portion and the posterior or neural portion with a small section dividing the lobes called the pars intermedia The two lobes have different structures and secrete completely different hormones



FIG 11

POSITION OF PITUITARY

The gland lies on the under side of the brain at the position marked by the circle

Anterior Lobe

The anterior lobe of the pituitary has been the subject of considerable research by physiologists and biochemists and many attempts have

been made to isolate the hormones which regulate the activity of other endocrine glands It is extremely difficult to isolate active fractions in a state of purity and opinion is divided on the number of different hor



Fig 12
Section of Ox Pituitary

mones produced by the gland The six given in Table III have each been obtained in preparations almost completely free from other forms of activity and are generally accepted as being well defined separate substances The existence of ketogenic parathyrotrophic glycotrophic diabetogenic and pancreatrophic substances has also been postulated but it seems likely that their effects are due to one or more of those hormones named in Table III[54]

Table III
Hor iones of the Anterior Lobe

Hormo	*Fu tion*	*Fi st Describ d*
Growth Hormone	N cessary for cor e t skel tal development	Evan and Long 1921
Gonadotroph c Hormones		
(1) Follicle St mulating Hormone	Cont ols develop n nt of Graafian foll cles	Fevold and Hisaw 1933
(2) Luteinis ng Hormon	Es ent al for product on of corpora lutea	Fevold and Hisa 1933 Wallan La rence 1934
(3) Luteot ophic Hormon *Lactogen c Horm ne* (P olactin)	Stimulates m lk secre tion *also conc rned* v *ith* the menstrual cycle and pregnancy	Riddle Bates and *Dyksl orn 1932*
Thyrotroph Hormone	Essential fo correct func t on ng of the thyro d gland	Loeb 1929 Aron 19 9
Adrenocorticotrophic Ho mone (Cort cotroph n)	Essential fo the correct funct oning of the ad en l cortex	Smith 1930

A photomicrograph of a section of the anterior lobe which has been stained with eosin and pyrrhol blue shows that three types of cells are present in the gland in varying proportions They are chromophobe cells which are only faintly stained with aniline dyes and chromophil cells which are divided into acidophil cells and basophil cells according to their staining properties Acidophil cells are stained by acid dyes such as eosin and basophil cells by basic dyes such as pyrrhol blue

The chromophobe cells appear to be the mother cells from which basophil and acidophil cells are derived Severinghaus[55] suggests that the specific granules in the different cells are precursors of different hormones The inactive chromophobe cells can change into hormonally active acidophil or basophil cells and this process can be reversed The changes in the proportion of each cell type can be interpreted as the result of the need for constant adaptation of the anterior lobe to different requirements of the body An examination of sections of the anterior lobe in pathological conditions may show an excessive growth of one type of cell for example in acromegaly a condition due primarily to hyperfunction of the anterior lobe there is hypersecretion of the acidophil cells or eosinophil adenoma of the pituitary A disease known as Cushing s syndrome is considered to result from a tumour of the basophil cells

Growth Hormone When the pituitary is removed from young animals growth ceases and Evans and Long[6] first showed that extracts of the anterior lobe contained growth promoting substances which when injected into rats produced animals of twice the normal size Highly purified preparations containing the growth hormone have been prepared and Li and Evans[57] claim to have isolated the hormone from the gland It is a water soluble protein which is easily destroyed by many physical and chemical reagents The growth hormone is essential for correct skeletal development and a deficiency resulting from an underactive pituitary produces dwarfism the dwarfs differ from cretins whose stunted growth is due to a deficient thyroid gland

An over production of the growth hormone of the anterior lobe produces persons of stature larger than the average If the overproduc tion occurs before the epiphyses of the bones are fully united the person becomes a giant but if it occurs after the bony epiphyses have united the result is acromegaly in which the features are coarse and the bones of the hands and feet are very large in proportion to the rest of the body

Anterior Lobe and the Gonads The earliest experiments on hypophysectomised animals showed that anterior lobe extracts greatly influence the gonads in males and females and during the last ten to fifteen years much work has been carried out on the gonad stimulating or gonadotrophic factors in the anterior lobe

There is an intimate interrelationship between the gonads of both

sexes and the anterior lobe and it has been shown that there is no difference in the hormones derived from the pituitary glands of males and females Although the existence of as many as eight different hormones capable of stimulating the sex glands has been postulated more recent work indicates that probably there are only two termed the follicle stimulating hormone (F S H) and the luteinising hormone (L H)

In the female the follicle stimulating hormone stimulates the ovary to produce follicles and secrete the œstrogenic hormone œstradiol The luteinising hormone stimulates the production of progesterone by the corpora lutea in the ovary after ovulation (see page 44) In the absence of the luteinising hormone luteinisation does not occur in hypophysectomised animals treated with follicle stimulating extracts[58] These two hormones must be present in the gland in the correctly balanced proportions and they play an essential part in the physiology of the reproductive system

In the male spermatogenesis and development of the testes are stimulated by the follicle stimulating hormone but it is the luteinising hormone which stimulates the formation of the male sex hormone testosterone which is produced by the interstitial tissue

TABLE IV

ACTION OF THE PITUITARY GONADOTROPHINS ON THE GONADS

Anterior Lobe

Follicle Stimulating Hormone		Luteinising Hormone	
Ovary	Testis	Ovary	Testis
Stimulates growth of ovarian follicle and secretion of œstrogen hormone œstradiol	Initiates and maintains spermatogenesis in seminiferous tubules	Stimulates luteinisation and secretion of corpus luteum hormone progesterone	Stimulates secretion of male hormone testosterone by interstitial tissue

Chorionic Gonadotrophin In 1928 Aschheim[59] showed that during pregnancy large amounts of gonadotrophic hormones appear in the urine and this is the basis of the best known diagnostic test for pregnancy (see page 46) It was concluded that the source of these hormones was the anterior lobe but further experiments showed that the purified extract from the urine called chorionic gonadotrophin did resemble but was not identical with the luteinising hormone It has now been shown that the origin of the gonadotrophic factors found in pregnancy urine is the chorionic cells of the placenta

Serum Gonadotrophin In 1930 Cole and Hart[60] observed that a follicle stimulating gonadotrophic substance was present in large

amounts in the blood of pregnant mares reaching a maximum con centration on about the seventieth day of gestation Boycott and Rowlands[61] investigated the biological nature of the extracts of pregnant women s serum and made the interesting observation that in contrast to the mares serum extract it possessed only luteinising properties similar to those of the extracts which had been prepared from preg nancy urine

Both chorionic gonadotrophin and serum gonadotrophin are avail able commercially in general chorionic extracts comprise mainly the luteinising hormone while serum gonadotrophin is mainly the follicle stimulating hormone



Fig 13
Action of Serum Gonadotrophin
Ovaries and uterus of immature rats before and after treatment with serum gonadotrophin

The synergistic action of the luteinising chorionic gonadotrophin from pregnancy urine and an extract containing the follicle stimula ting hormone of the anterior lobe has been investigated Evans Meyer and Simpson[62] observed that a combination of the two hormones produced a greater increase in ovarian size and weight than could be obtained by administering the components separately A standardised product is available and several reports indicate that it has a place in hormone therapy[63 64 65]

Anterior Lobe and Other Glands It has been shown by many workers that a close interrelation exists between the anterior lobe of the pituitary and other glands including the thyroid adrenals mammary glands and pancreas Purified extracts of the anterior lobe containing the thyrotrophic hormone induce hyperplasia of the thyroid and stimulate the liberation of an abnormal amount of thyroxine[66] The effects of their administration to experimental animals have been investigated[67 68 69], but few clinical reports are available Marx *et al* [70] suggest that the thyrotrophic hormone acts synergistically with the

growth hormone and consider that it may be of value in the treatment of pituitary dwarfism

Extracts from the anterior lobe produce hypertrophy of the adrenal cortex The active principle called the adrenocorticotrophic or corticotrophic hormone (ACTH) has been obtained in a pure form[71] and shown to control the activity of the adrenal cortex (see page 38)

The existence of a specific hormone in the anterior lobe which influences the growth of the mammary gland during pregnancy and is responsible for the secretion of milk has been confirmed and is known as the lactogenic hormone or prolactin[72, 73] It is also known as the luteotrophic hormone because it assists in maintaining the corpus luteum produced by the action of the luteinising hormone

Posterior Lobe

Extracts of the posterior lobe of the pituitary can be separated into two fractions one containing an oxytocic principle which causes a contraction of the uterus (the word oxytocic is derived from Greek words meaning rapid childbirth) and one consisting mainly of a substance which causes a rise in blood pressure under certain conditions and which has also an important antidiuretic effect reducing the amount of urine excreted The former principle is called oxytocin and the latter vasopressin

Verney[74] showed in experiments on the isolated kidney which is perfused with blood that the urine excreted was very dilute and resembled that obtained from a patient with diabetes insipidus When an extract of the posterior lobe was added to the perfusion fluid the kidney recovered its power to excrete a concentrated urine Verney also showed that the polyuria following isolation of the mammalian kidney was due to the removal of this organ from the normal sustained antidiuretic action of the pressor fraction of the posterior lobe

Injection of extracts of the posterior lobe produces a direct action on the capillaries which contract causing pallor of the skin The action of the extracts on the blood pressure varies with different animals Generally there is no significant rise in blood pressure there may be a momentary fall succeeded by a more prolonged increase in the pulse rate Extracts of the posterior lobe have been used to raise the blood pressure after shock If the posterior lobe of the pituitary is damaged by disease or injury the hypothalamic syndrome may develop when the individual becomes exceptionally sleepy and emotional control is upset The normal water balance is impaired because the renal tubules are no longer able to re absorb water from the urine and to make up for the large volume of water lost equally large volumes must be imbibed

Pars Intermedia The posterior lobe of the pituitary gland includes the pars intermedia the small portion of the gland dividing

the two lobes In man its function is unknown but it is well developed in fishes amphibia and reptiles Frogs can change their colour to make *themselves less conspicuous* and this is due to an alteration in the pigment cells which are stimulated by the hormone of the pars intermedia The pigment cells melanophores or black cells are expanded and dispersed

Adrenals

The adrenal or suprarenal glands are situated on the upper surface of the kidneys and are composed of two different tissues an outer layer or cortex and an inner part or medulla



Fig 14
Position of Adrenal
The adrenal (A) lie on the upper surface of the kidney (*B*)
The photograph also shows the connecting nerve plexus (*C*)
and the aorta (*D*)

The adrenal glands have a particularly rich blood supply which is an indication of the importance of these organs and they secrete groups of hormones with widely differing actions

Adrenal Medulla In 1894 Oliver and Schafer[75] showed that injection of an extract of the adrenal medulla produced a remarkable rise in blood pressure and later workers succeeded in isolating from the gland a crystalline compound which they called epinephrine The active principle obtained in pure form in 1901 by Takamine[76] and others was marketed as Adrenalin which is a Latin rendering of the Greek term epinephrine and it is now known as adrenaline except in

the United States of America where it is still called epinephrine Adrenaline was the first hormone to be isolated in crystalline form

Unlike the cortex the medulla is apparently not essential to life and in spite of extensive studies on its physiological function its action in persons under normal conditions is unknown It has been suggested that adrenaline acts as an emergency hormone because it is secreted in increased amounts in times of physical or emotional stress and enables the body sometimes to perform seemingly impossible tasks This theory has not been substantiated by experiments on adrenalectomised animals but it has been shown that the barking of a dog increases the rate of a cat s denervated heart



FIG 15

SECTION OF ADRENAL

From the fibrous capsule numerous trabeculæ containing blood sinuses run at right angles into the wh te fibrous and yellow fatty tissues of the cortex divid ng t into columns of cells The dark central portion the medulla is red and r chly suppl ed v ith blood

Action of Adrenaline Adrenaline is a sympathomimetic drug that is its effects in the body are comparable to those produced by stimulating the sympathetic nervous system Other compounds chemically related to adrenaline have been investigated by Barger and Dale[77] and the pharmacological action and therapeutic uses of related compounds are described by Gunn[78] Slight changes in the chemical constitution may produce considerable variations in pharmacological activity

Adrenaline on injection produces many complex physiological effects in the body and has many therapeutic uses its action is immediate and its effect transient Perhaps its most important action is the

raising of the blood pressure adrenaline is the most potent vasopressor drug known and if given intravenously or directly into the heart muscle it produces a sudden rise of blood pressure lasting for a few minutes due mainly to its constricting effect on the blood vessels

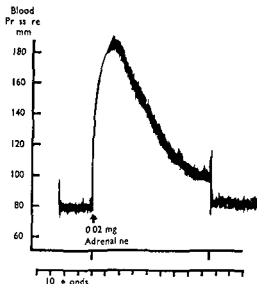


FIG 16

ACTION OF ADRENALINE

Graph showing the rapid rise in blood pressure after njecting adrenaline into a rabbit

Adrenaline relaxes the bronchial musculature and is therefore used *extensively in the prophylaxis and treatment of asthmatic attacks In* addition it dilates the pupils of the eye and inhibits movement in the uterus intestines and bladder Adrenaline plays an important part in carbohydrate metabolism it stimulates the conversion of liver glycogen to blood sugar and can be considered to have an antagonistic action to insulin Cori and Cori [9] consider that adrenaline accelerates the cycle of muscle glycogen to liver glycogen while insulin accelerates that of blood glucose to muscle glycogen leading to hypoglycæmia and depletion of liver glycogen

Action of Noradrenaline Noradrenaline which differs from adrenaline by the absence of a methyl group from the nitrogen atom was first synthesised by Stolz[80] in 1904 but its presence in the adrenal gland was not reported until 1949 when Holton[81] showed that tumours of the medulla contained large amounts of noradrenaline The presence of noradrenaline in the adrenal medulla in addition to adrenaline has

now been established[8] [83] and pharmacological and clinical studies have shown that noradrenaline exerts a more powerful effect on the heart than adrenaline It is believed that noradrenaline is a precursor of adrenaline and that essential hypertension results from failure of the body to effect methylation Noradrenaline has been shown to be effective in the treatment of certain cases of acute hypotension[84]

Adrenal Cortex The cortex or outer layer of the adrenal gland is essential to life and the effects of adrenal cortical deficiency a clinical example of which is Addison s disease are marked disturbances in the metabolism of electrolytes carbohydrates proteins and fats associated with loss of appetite general muscular weakness decreased resistance to cold low blood pressure loss of weight and vomiting In man there is pigmentation of the skin chiefly on exposed areas but this does not occur in experimental animals Some of these symptoms can be controlled by the administration of sodium chloride by mouth or by the injection of normal saline solution and a diminished intake of potassium salts but replacement therapy with adrenal cortical extracts or synthetic deoxycortone acetate which does not have exactly the same action as the natural extract is usually employed Overactivity of the adrenal cortex frequently due to a tumour leads to a condition known as adrenal virilism which is characterised by precocious growth and sexual maturity in females there is masculinisation

An important function of the adrenal cortex is the maintenance of correct electrolytic balance or relative concentration of sodium and potassium salts in the body An underactive gland gives rise to an increased excretion of sodium followed by a fall in the serum chloride and bicarbonate accompanied by a rise in the concentration of potassium in the extracellular fluids and its retention in the body cells It is possible to prolong the lives of adrenalectomised animals by the administration of sodium chloride alone The adrenal cortex also affects water metabolism After adrenalectomy there is a reduction in the diuretic response to water and a tendency to water intoxication The administration of adrenal cortical extracts in man leads to profuse diuresis

Twenty eight crystalline steroids have been isolated from the cortex In 1934 Kendall *et al* [85] isolated a crystalline substance capable of maintaining the life of adrenalectomised animals In 1936 Reichstein[86] obtained from the adrenal cortex a sterol with androgenic properties *which he named androsterone Ingle[87] isolated seven compounds which* were effective in relieving different conditions associated with adrenal cortical deficiency one of the most active being deoxycortone

Adrenal Cortex and Other Glands The hormones of the adrenal cortex and the gonads are closely related chemically (see page 77) and adrenal cortical insufficiency profoundly affects the

secondary sex characteristics Speert[88], by injection of deoxycortone acetate has produced changes in the ovary similar to those produced by the ovarian hormones Other workers have separated œstrogenic and progestational substances from adrenal cortical extracts

Many relationships between the adrenals and the other endocrine glands have been postulated and although a considerable amount of experimental work has been carried out it is not yet clear to what extent the glands are physiologically related It is known that the cortex is concerned with carbohydrate metabolism (see page 26) The adreno corticotrophic hormone of the anterior lobe of the pituitary stimulates the adrenal cortex and conversely adrenal cortical deficiency may be accompanied by dysfunction of the anterior lobe of the pituitary In some syndromes for example Cushing s syndrome there may be either an adrenocortical or an anterior pituitary tumour

There are some factors common to both the adrenals and the gonads The administration of œstrogenic compounds causes hypertrophy of the adrenals Androgens on the other hand either have no effect on the size of the adrenals or produce atrophy of the glands Castration in the male animal is followed by adrenal cortical hypertrophy

The work of Selye[89] shows that the adrenal cortex plays an important part in the protection of an animal from environmental stress He has further shown that this process of adaptation to stress may be accom panied in man by pathological conditions which he calls diseases of adaptation and which include rheumatoid arthritis and hypertension

On this theoretical basis research was carried out in Germany during the Second World War on the administration of adrenal cortical hor mones to air crews as a means of protection from the great strain of high altitude flying Of even greater medical importance is the discovery by Hench and his colleagues[90] that cortisone administered in a dosage of 100 milligrams a day will rapidly alleviate the symptoms of rheuma toid arthritis and rheumatic fever The adrenocorticotrophic hormone of the anterior lobe of the pituitary has been shown to give similar beneficial results in the rheumatic diseases

Gonads

The gonads or sex glands comprise the ovaries in the female and the testes in the male and they produce hormones which greatly influence the body as a whole Their main function is concerned with reproduc tion but they are also determining factors in the personal make up both physical and psychological

Before discussing the hormones secreted by the gonads consideration should be given to the anatomy of the male and female reproductive systems In the embryo during the early weeks the gonad is morpho logically a bisexual organ which may develop into either an ovary or a testis depending on the predominant hereditary sex characteristics

Male Reproductive System The male reproductive system consists of the testes the prostate gland lying below the bladder the seminal vesicles and Cowper s glands the secretions of which are added to the spermatozoa The testes contain convoluted seminiferous tubules which produce spermatozoa and in between these tubules there are groups of interstitial cells the source of the male hormone testosterone

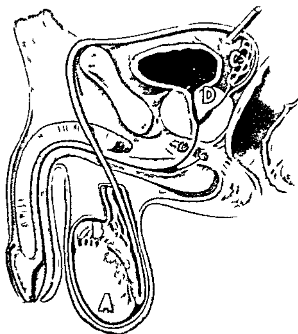


Fig 17
Male Sex Organs
(*A*) Testis (*B*) Ep d dymis (*C*) Seminal ves cle (*D*) Prostate

Spermatozoa are transferred from the seminiferous tubules to the epididymis an exceedingly tortuous tubule about 6 metres in length on the posterior surface of the testis Here they are stored and mature The production of spermatozoa and testosterone usually continues throughout life and there is so far as is known no periodic alteration in the amount of hormone secreted analogous to the menstrual cycle in the female There is probably a diminution in older men a male climacteric having been described which may be relieved by replacement therapy An extract from bulls testes when injected into capons produces growth of the comb wattles and ear lobes The effect of

injections of different androgens and also of œstrogens on capons has been the subject of many investigations and it is a convenient test for assessing the potency of different hormone preparations

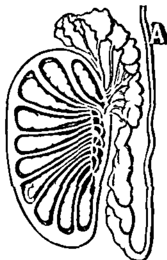


Fig 18

Testis

Diagram showing the relation between the vas deferens (A) the epididymis (B) and the convoluted tubules of the testis (C)



Fig 19

Effect of Testosterone on Capon's Comb

Left before administration of testosterone *right* after administration

The effects of castration have been known for centuries There is a profound alteration in somatic growth the bones become longer the muscles are less developed and fat is deposited within the muscles When castration in the human male is performed before puberty the high pitched voice is retained and the boy does not develop typical

male characteristics such as the normal distribution of hair If it is performed after puberty the effects are less marked and secondary male characteristics can be restored by the injection of testosterone

The testes are closely related physiologically to the anterior lobe of the pituitary gland (see page 30) and if the pituitary gonadotrophic hormones are imperfectly secreted development of the testes is retarded and their function diminished

Perhaps the most typical sex characteristic which is influenced by the male hormone is the distribution of hair and it is interesting to note that the pattern of distribution of head hair in men and women may be determined by the hormones produced by the gonads Hamilton[11] suggests that baldness is an hereditary trait requiring a physiological level of androgen for its development it is not produced by androgens in individuals not carrying the necessary hereditary factors The colour of the skin distribution of subcutaneous fat depth of the voice skeletal growth and greater muscular strength of the male are secondary sex characteristics resulting from the action of the male hormone

Female Reproductive System The essential organs of reproduction in the female are the two ovaries ovoid organs each weighing about 5 grammes They are suspended in the pelvis and are in contact with the uterine or fallopian tubes which are about 10 centimetres long The ovaries during the period of reproductive life alter rhythmi

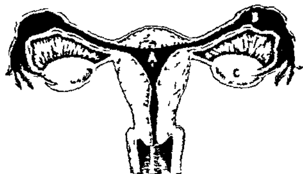


Fig 20
Female Sex Organs
(A) Uterus (B) Fallopian tube (C) Ovary

cally in structure and perform a dual function the production of ova and of two hormones oestradiol and progesterone which are necessary for the functioning of the female cycle and for the maintenance of pregnancy

At birth the ovaries contain from 30 000 to 400 000 ova The number of ova progressively diminishes throughout life from 400 to 500 attaining maturity usually one at monthly intervals Each ovum is carried through the fallopian tube to the uterus If fertilisation takes place it occurs in the fallopian tube and the fertilised ovum is then transferred to the uterus If fertilisation does not occur the ovum degenerates and is expelled during menstruation

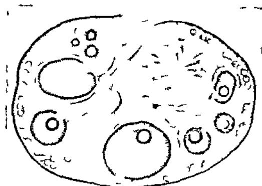


FIG 21
SECTION OF OVARY (× 2)
The ovary consists of a mass of stroma covered by germinal epithelium which contains primordial ova Graafian follicles enclosing ova at various stages of maturation corpora lutea blood vessels lymphatics and nerves One follicle (*top left*) has burst and expelled it ovum The larger non nucleated mass of an old follicle (*top right*) has developed into a corpus luteum

The uterus or womb is a thick walled muscular organ with an internal mucous lining (endometrium) a muscular coat (myometrium) and an external peritoneal covering The lower end of the uterus becomes narrower and leads to the vagina the passage from the uterus to the exterior of the body If an ovum is fertilised it becomes embedded in the wall of the uterus and is normally retained there until pre natal development is completed

Reproductive Cycle in Mammals At maturity the female reproductive system in mammals undergoes regular periodic changes In most mammals it is usual to divide the cycle into four stages —

(*a*) pro œstrus when the follicle ripens
(*b*) œstrus the period of heat when ovulation occurs
(*c*) metœstrus the period when retrogressive changes occur in the ovary
(*d*) diœstrus or anœstrus the period of sexual inactivity

In 1917 Stockard[9] demonstrated that there were characteristic changes in the cells of the vaginal epithelium of animals during the œstrus cycle These changes can be followed by a microscopic examination of vaginal smears taken at different phases of the cycle which forms the basis of much experimental investigation of the ovarian hormones and their artificial analogues (see page 133)

During diœstrus vaginal smears consist mainly of leucocytes with few nucleated cells During pro-œstrus leucocytes are absent and a large number of nucleated epithelial cells are present During œstrus the epithelial cells lose their nuclei and non nucleated cornified cells are found in large numbers Vaginal smears taken during metœstrus show many leucocytes with some epithelial cells

Human Reproductive or Menstrual Cycle The human female reproductive cycle or menstrual cycle is different from the œstrus cycle in lower mammals It is however dependent on the production by the ovary of the correct amounts of hormones at regular times and also on the correct balance between the gonadotrophic hormones of the anterior lobe of the pituitary and the ovarian hormones The cycle in humans normally occupies twenty-eight days and recurs regularly from puberty until the menopause when declining reproductive activity finally ceases

Changes in the Uterus As the menstrual cycle is a continuous building up shedding and repairing of the endometrial lining there is no clear cut division between one stage of the process and the next The cycle may however conveniently be divided into three main phases —

(1) A bleeding phase or period of menstruation characterised by a degeneration of the endometrium and occupying approximately four days

(2) A proliferative or pre-ovulatory phase lasting seven to ten days in the later stages of which the epithelium of the uterine mucosa develops and increases in thickness the cells at the surface of the epithelium becoming more columnar From the fourth to the eighth day of the cycle the tissue is resting and recovering from the effects of menstruation From the eighth to the thirteenth day under the stimulation of the œstrogenic hormone regeneration occurs On the basal layer of the inner surface of the uterus a gradually thickening layer of glandular tissue the endometrium develops reaching its maximum thickness by about the fifteenth day This layer consists of straight tubular initially inactive glands separated by connective tissue At about the fourteenth day a Graafian follicle in the ovary ruptures and liberates an ovum which may or may not later be fertilised

(3) A progestational or post-ovulatory phase lasting for approximately fourteen days after ovulation when the glands undergo characteristic changes becoming twisted and spiral instead of straight

The cells lining the glands begin to elaborate glycogen and the connective tissue changes to form characteristic polyhedral decidual cells During this phase the endometrium is highly vascular and is ready to receive and implant a fertilised ovum which if present, becomes embedded in the vascular endometrial tissue A placenta is ultimately built up and the ovarian membranes develop round the growing fœtus If however the liberated ovum is not fertilised the uterine mucosa again slowly degenerates and menstruation occurs The period occupied by each phase of the cycle as described above is only an average In practice considerable variations occur in the timing of the cycles of different women and even of the same woman

Changes in the Ovaries Concurrently with these changes in the uterus cyclic changes are taking place in the ovaries A primordial follicle of one of the ovaries becomes distended with fluid (liquor folliculi) The swollen follicle on attaining maturity bursts at the surface of the ovary and the ovum passes into the fallopian tube After the discharge of the ovum changes occur within the ruptured ovarian follicle resulting in the formation of a glandular structure the cells of which become filled with a yellow fat lutein from which the name corpus luteum (yellow body) is derived and the hormone progesterone is secreted Progesterone is essential for —

(*a*) The development of a premenstrual endometrium
(*b*) Maintenance of pregnancy
(*c*) Growth of the lobules of the breast
(*d*) Inhibition of ovulation during pregnancy

Action of the Anterior Lobe of the Pituitary The cyclic changes in the ovary resulting in the maturation of an ovum which is discharged and the growth of the corpus luteum in the ruptured follicle are controlled by the gonadotrophic hormones of the anterior lobe of the pituitary gland Thus the follicle stimulating hormone stimulates the production of the ovarian hormone œstradiol which is secreted in increasing amounts from the fourth to the twenty eighth day of the cycle and the luteinising hormone stimulates the production of the corpus luteum hormone progesterone which is secreted in increasing amounts from the fourteenth to the twenty eighth day of the cycle

There is some doubt as to how the two ovarian hormones and the two pituitary gonadotrophic hormones act in the establishment of the menstrual cycle but it is known that a definite balance exists and must be maintained between the hormones elaborated by the different glands The amount of œstrogens and progesterone present in the ovaries cannot be measured directly but an estimation of the excretion products is of importance in diagnosis and treatment

Excretion of Progesterone In the normal menstrual cycle progesterone is eliminated in the urine as an inactive water soluble compound

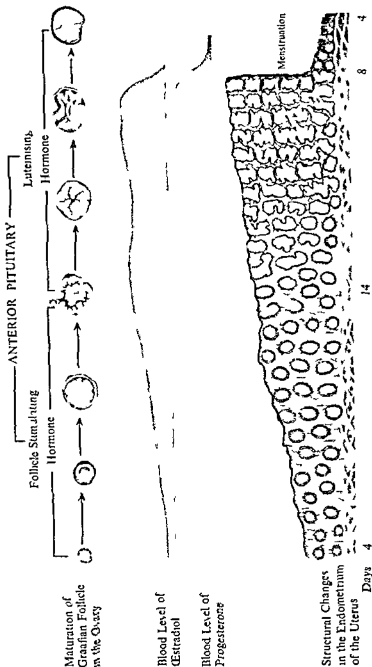


FIG 22

DIAGRAMMATIC REPRESENTATION OF NORMAL MENSTRUAL CYCLE

sodium pregnanediol glycuronidate and the excretion of this pregnanediol closely parallels *the production of progesterone by the corpus luteum* Excretion begins about twelve days before the onset of menstruation (a day or two after ovulation) and reaches a peak about

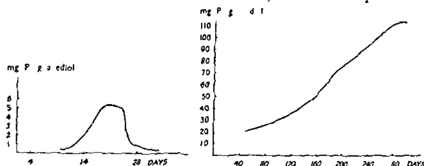


Fig 23

Pregnanediol Excretion

The left hand chart shows the daily excretion during the menstrual cycle The right hand chart shows the very much larger daily excretion during pregnancy

one week before menstruation The maximal daily excretion is approximately 5 milligrams During pregnancy however the output of pregnanediol increases considerably from the seventieth to the ninetieth day and may reach 60 to 100 milligrams daily by the ninth month Probably most of the progesterone produced after the third month of pregnancy *is made by the placenta and not the corpus luteum and failure of the* placenta to produce progesterone may be regarded as one of the reasons for abortion

Pregnancy Diagnosis

During pregnancy œstrogens progesterone and the gonadotrophic hormones of the anterior lobe of the pituitary gland are excreted in increased amounts and the presence of the chorionic gonadotrophic hormone in the urine forms the basis of biological tests for the existence of pregnancy

Aschheim Zondek Test In the Aschheim Zondek test[93] 0 5 millilitre of a morning sample of urine is injected subcutaneously twice *daily for three consecutive days into each of six immature female* white mice 3 to 4 weeks old and weighing 6 to 8 grammes The animals are killed 100 hours after the first injection and their ovaries examined If the ovaries are larger than normal and have large follicles and corpora lutea some of which may show hæmorrhagic spots (blood points) the test is positive

Fried t A rapid and reliable modification of the
Aschh *t was made by Friedman[94, 95] who discovered*

that a single intravenous injection of 8 to 15 millilitres of pregnancy urine usually induced ovulation within forty eight hours in young mature non pregnant female rabbits Unlike most other animals the rabbit does not ovulate spontaneously and the test is positive when the ovaries at autopsy show the presence of recently ruptured follicles with hæmorrhagic areas

Xenopus Test In 1930 Hogben[96] showed that extracts of the anterior lobe of the pituitary induced ovulation in the hypophysectomised mature South African claw toed frog (*Xenopus lævis*) and this fact was subsequently made use of in developing the xenopus pregnancy test sometimes referred to as Hogben s test

The mature female xenopus carries eggs throughout the year but extrudes them only after mating or under laboratory conditions after the injection of chorionic gonadotrophin this test is of interest in that it is comparatively simple to carry out no operation is required and the result is known in from four to eighteen hours The frogs are kept in tanks of water at 70 F and are fed on liver beef heart and earthworms 1 millilitre of an extract of the urine is injected subcutaneously into the dorsal lymph sac of the frog which is returned to the tank and observed at intervals after four hours for the extrusion of eggs If the test is positive eggs usually appear in from six to twelve hours if none appears at the end of eighteen hours the test is negative Reliable results can be expected in 96 to 99 per cent of cases and are first demonstrable about ten days after the first missed menstruation

Male Toad Tests Galli Mainini[97] used the male toad *Bufo arenarum* as the test animal Usually 10 millilitres of the patient s urine is injected subcutaneously and after a few hours a drop of urine is collected from the toad and examined for the presence of spermatozoa Good results have also been obtained using English male toads (*Bufo vulgaris*)[98] several South American toads and the frog *Rana pipiens*[99]

In all these tests false positives may be obtained in patients with hydatidiform mole and chorionepithelioma but false negatives rarely or never occur Hydatidiform mole occurs when the ovum fails to develop and there is abnormal growth of the tissues concerned in the formation of the membranes which normally surround the developing embryo Chorionepithelioma is a form of malignant disease arising from the placental relics of a former pregnancy or of a hydatidiform mole

Guterman Test This test[100] consists essentially of the extraction with toluene of the acid hydrolysed pregnanediol complex of urine followed by the purification and precipitation of the pregnanediol Ten millilitres of concentrated sulphuric acid is added to the precipitate from 100 millilitres of a morning specimen of urine and the colour

is observed in a test tube when solution is complete Colourless to light yellow is taken as negative deep yellow to deep orange brown as positive

Conditions which are associated with the excretion of luteinising hormone such as hydatidiform moles and testicular tumours give negative results by this technique which therefore has the advantage of distinguishing between normal placental and abnormal placental or placental like tissue

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CHAPTER III

CHEMISTRY OF THE NON STEROID HORMONES

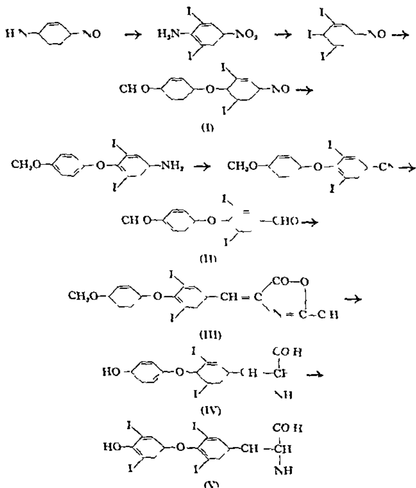
THE non steroid hormones comprise those which are derived from the thyroid parathyroids pancreas pituitary (both posterior and anterior lobes) and adrenal medulla The hormones associated with the para thyroids pancreas and pituitary possess the chemical and physical characteristics of proteins and with the exception of insulin little is known of the details of their chemical constitution On the other hand the hormone of the thyroid thyroxine and those of the adrenal medulla adrenaline and noradrenaline are relatively simple chemical com pounds of known constitution

Thyroid

The isolation of the active principle from the thyroid gland was first achieved by Kendall[1] but the method of isolation was subsequently modified and improved by Harington[3] Since the hormone is present in the form of a peptide in the protein iodothyroglobulin hydrolysis has to be carried out firstly with aqueous sodium hydroxide and sub sequently with aqueous baryta In Harington s method hydrolysis is carried out with hot baryta using successively a 10 per cent solution and a 40 per cent solution In this manner the hormone is isolated as the barium salt which may be decomposed by addition of a slight excess of aqueous sodium sulphate to its suspension in hot 1 per cent aqueous sodium hydroxide After filtration from barium sulphate the *free thyroxine may be isolated by acidification Subsequent purification* is effected either by dissolving the crude product in alcoholic alkali followed by acidification with acetic acid or by crystallisation of the *sodium salt Thyroxine obtained by this method is optically inactive* but by utilising a process of enzymic hydrolysis Harington later ob tained L thyroxine m p 235 $[\alpha]_{5461} - 3\ 8$ (sodium salt in 60 per cent ethyl alcohol)[4]

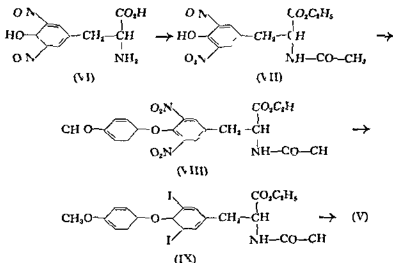
Thyroxine ($C_{15}H_{11}O_4NI_4$) Thyroxine (V) is a white crystalline compound which is sparingly soluble in water and insoluble in organic solvents It dissolves in aqueous ammonia and in alcoholic alkalis The constitution of thyroxine was established by Harington[5] who was able to replace the iodine in the molecule by hydrogen by shaking an alkaline solution of the hormone *with colloidal palladium in the presence of* hydrogen The resulting compound $C_{15}H_{15}O_4N$ known as thyronine was shown to be an amino acid and its constitution was proved by synthesis *Finally the synthesis of thyroxine* itself was reported by Harington and Barger in 1927[6] In this synthesis *p* nitraniline was converted into 2 6 diiodo 4 nitroaniline and then by means of the diazo

reaction into 3 4 5 triiodonitrobenzene Condensation of the latter with the monomethyl ether of hydroquinone in methyl ethyl ketone solution in the presence of potassium carbonate gave the diphenyl ether (I) in which the nitro group was subsequently reduced and replaced by the cyano group by means of the Sandmeyer reaction Reduction of the cyano group with anhydrous stannous chloride gave the aldehyde (II) which was condensed with hippuric acid in order to introduce the alanine side chain The azlactone (III) which was first formed was hydrolysed and reduced and then demethylated and debenzoylated to give the diiodo acid (IV) The introduction of the additional iodine atoms was effected by the addition of a solution of iodine in potassium iodide to a solution of the ammonium salt Liberation of the free acid on acidification gave DL thyroxine (V)

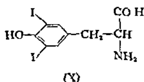


The resolution of the racemic compound presented considerable difficulties owing to its sparing solubility but in 1928 Harington[7] was able to resolve DL 3 5 diiodothyronine (IV) into the two optically active forms into each of which the two additional iodine atoms were subsequently introduced

An improved synthesis of thyroxine has recently been reported[8] and an extension of this method to L tyrosine leads to L thyroxine without loss of optical activity[9] In this process L tyrosine is converted to 3 5 dinitro L tyrosine (VI) which is submitted to acetylation and esterification to give the ester (VII) Reaction with the monomethyl ether of hydroquinone in pyridine gives 3 5 dinitro 4 p methoxy phenoxy N acetyl L phenylalanine ethyl ester (VIII) which is then reduced to the diamine and submitted to the Sandmeyer procedure to give the diiodo ester (IX) Subsequent hydrolysis and iodination with iodine in ethylamine solution gives L thyroxine (V) ($[\alpha]_D - 5\ 7$ in N ethyl alcoholic sodium hydroxide) The overall yield from L tyrosine is 26 per cent



In addition to thyroxine the thyroid gland contains 3 5 diiodotyrosine (X)[10] an amino acid which had been previously isolated from coral This amino acid is regarded as a precursor in the biogenesis of thyroxine By the use of radioactive iodine as a tracer it has been shown that iodine administered as iodide becomes located in the thyroid gland being incorporated firstly in 3 5 diiodotyrosine and finally in the thyroxine component of the protein iodothyroglobulin In 1939 Ludwig and von Mutzenbecher[11] obtained physiologically active compounds



by the iodination of casein and similar proteins and it has been shown that in these reactions 3 5 diiodotyrosine and thyroxine are formed[12, 13] The direct conversion of 3 5 diiodotyrosine into thyroxine was also established experimentally by von Mutzenbecher[14] and by Harington[15]

PARATHYROIDS

The hormone of the parathyroids is a protein which is decomposed by trypsin It is therefore not active when given by mouth Potent extracts of the parathyroids have been prepared independently by Collip[16] by Tweedy[17] and by Dyer[18] by boiling the fresh glands for a limited period of time with dilute acid Little is known of the chemistry of the active principle

PANCREAS

The secretion of those portions of the pancreas termed the islets of Langerhans is the hormone insulin

Insulin Insulin can be isolated from the pancreas of the ox or pig and the islets derived from the pancreas of certain fish also provide a useful source The hormone possesses the normal characteristics of a protein and it was thus difficult to obtain in crystalline form Various processes have been used for the isolation of the hormone and the following method due to Scott and Parker[19] is typical The fresh minced pancreas from the ox is allowed to stand overnight in acidified aqueous alcohol The extract is separated in a centrifuge and made alkaline with ammonia The inactive precipitate is filtered off the filtrate acidified with sulphuric acid and the alcohol removed under reduced pressure The fats which separate are filtered off and the active principle is liberated from the filtrate by the addition of salt The precipitated material is further purified by repeated carefully controlled precipitation from solution The original literature should be consulted for further details of this and other processes of isolation and purification Obtained in this manner insulin is an amorphous powder but Abel in 1926[20] and Harington and Scott in 1929[21] reported the preparation of crystalline insulin by precipitation from a highly buffered solution in the presence of either large quantities of brucine acetate and pyridine or saponin The crystals (see Fig 24) were found to contain zinc[22, 23] but the exact manner in which the zinc is combined is uncertain and contrary to earlier reports the percentage of zinc is not strictly constant[24, 25] Crystalline insulin turns brown at 216 and melts with decomposition at 233 It is optically active and lævorotatory The iso electric point is at pH 5 3 but the best conditions for crystallisation are probably within the range pH 5 8 to 6 3 As an amphoteric substance insulin is soluble in both acids and alkalis but it is not stable in alkaline solution Salts such as the hydrochloride sulphate and picrate can be obtained as amorphous powders It gives the normal reactions

for proteins such as the biuret Millon ninhydrin and xanthoproteic tests and contains about 15 per cent of nitrogen and about 3 3 per cent of sulphur It is sensitive to proteolytic oxidation and reduction enzymes The molecular weight of insulin has been given as 20 000 by chemical methods but the ultracentrifuge method gives values of 46 000 to 48 000 and the X ray method a value of 36 000 Viscosity measurements give values between 40 000 and 50 000 The unit cell

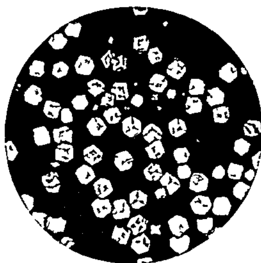


FIG 24
INSULIN CRYSTALS (×200)

from X ray measurements was shown to possess trigonal symmetry which indicated a three unit structure with a molecular weight of 12 000 for each unit Gutfreund[26] considers that the real molecular weight of insulin is 12 000 and that in neutral solution four such molecules are loosely bound together into a large molecule of molecular weight 48 000 which dissociates on dilution or at extreme *p*H values In the crystalline form three such molecules are combined in the unit cell On the basis of a molecular weight of 12 000 coupled with the results of the application of modern methods of amino acid analysis Sanger[27] has given the figures in Table V for the number of the various amino acid units in the insulin molecule

This analysis accounts for about 98 5 per cent of the insulin molecule The manner in which these units are arranged in the molecule is largely unknown although the presence of certain amino acid sequences has been demonstrated All attempts to separate from insulin an active compound of comparatively low molecular weight have been unsuccessful[28, 29, 30] and even mild hydrolysis results in loss of activity

TABLE V

AMINO ACIDS IN THE INSULIN MOLECULE

Amino-acid	*Number of Units per Molecule*
Glutamic Acid	15
Leucine	12
Cystine/2	12
Amide N	12
Tyrosine	9
Valine	8
Glycine	7
Alanine	7
Phenylalanine	6
Serine	6
Aspartic Acid	6
Histidine	4
Proline	3
*iso*Leucine	3
Arginine	2
Lysine	2
Threonine	2

While it has been shown that the presence of certain groups in the molecule is essential for activity and that the presence of others is not it remains true that the specific activity of insulin must be regarded as a property of the molecule as a whole or of some labile degradation product which has so far escaped detection

Insulin cannot be administered by mouth owing to its destruction by proteolytic enzymes Many successful attempts have been made to obtain derivatives which give rise to a more prolonged action after injection Hagedorn[31] showed that insulin can be combined with other substances to give products which have an iso electric point nearer to that of tissue fluids than has insulin itself In this way active compounds of lower solubility are obtained Compounds or mixtures of insulin with saponins bile acids and blood serum have been recommended Among the more successful substances of this type may be mentioned protamine insulin which is a combination of insulin hydrochloride with protamine obtained from the sperm of rainbow trout This substance is more slowly absorbed and a further improvement can be obtained by the addition of a small quantity of zinc chloride[3, 33] (see pages 23 and 177)

PITUITARY

The pituitary gland or hypophysis consists of two parts the posterior lobe and the anterior lobe which in nearly all animals are assembled together in one organ In the whale the two parts are anatomically independent

Posterior Lobe At least two hormones are produced in the posterior lobe Of these one causes the contraction of the uterus and

for proteins such as the biuret Millon ninhydrin and xanthoproteic tests and contains about 15 per cent of nitrogen and about 3 3 per cent of sulphur It is sensitive to proteolytic oxidation and reduction enzymes The molecular weight of insulin has been given as 20 000 by chemical methods but the ultracentrifuge method gives values of 46 000 to 48 000 and the X ray method a value of 36 000 Viscosity measurements give values between 40 000 and 50 000 The unit cell

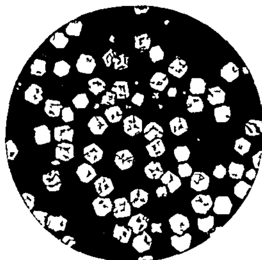


FIG 24
INSULIN CRYSTALS (×200)

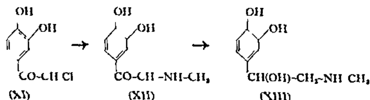
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the alcoholic filtrate is concentrated Addition of concentrated ammonia to the residual aqueous solution liberates free adrenaline which may be purified by solution in an alcoholic solution of oxalic acid filtration dilution with water and addition of ammonia Owing to the susceptibility of adrenaline to oxidation it is essential to carry out the extraction processes under reduced pressure or in an atmosphere of carbon dioxide or under a layer of light petroleum Owing to the simplicity of the process the natural product continues to be used in considerable quantities

Adrenaline ($C_9H_{13}O_3N$) Adrenaline (XIII) is a colourless crystalline compound m p 211 which is almost insoluble in water and most organic solvents but dissolves readily in acids and alkalis It is thus an amphoteric substance which displays both basic and acidic properties It is comparatively stable in acid solution but in the presence of alkalis it is readily oxidised It gives a green colour with aqueous ferric chloride solution and possesses strong reducing properties The natural hormone is lævorotatory ($[\alpha]_D$ -53 in aqueous hydrochloric acid) The racemic form is less active physiologically since (+) adrenaline has only one twelfth to one sixteenth the activity of the (−) isomeride

Adrenaline (XIII) is a derivative of catechol containing a secondary alcohol group and also a methylamino group Evidence for this constitution was obtained by degradation experiments and the constitution was confirmed by synthesis which was achieved almost simultaneously by Dakin[41] and by Stolz[42] 3 4 Dihydroxy-ω-chloroacetophenone (XI) was prepared by the action of chloroacetic acid on catechol in the presence of phosphorus oxychloride a reaction which was subsequently investigated and improved by Ott[43] By the action of an excess of a concentrated aqueous solution of methylamine on the chloro-ketone suspended in alcohol 3 4 dihydroxy ω methylaminoacetophenone (XII) was obtained and this on reduction gave adrenaline (XIII) Reduction can be effected either electrolytically by means of aluminium amalgam or catalytically with hydrogen and palladium The



resulting (±) adrenaline can be resolved by means of its bitartrate (+) adrenaline (+) tartrate being much more soluble in methyl alcohol than (−) adrenaline (+) tartrate[44] Further purification of (−) adrenaline (+) tartrate is effected by crystallisation from 95 per cent methyl alcohol The crude (+) adrenaline (+) tartrate is

the other constriction of the capillaries and contraction of the intestines Both extracts have an antidiuretic effect and are used in the treatment of diabetes insipidus These hormones are proteins

Anterior Lobe The anterior lobe of the pituitary is a gland of the greatest importance which has far reaching influences on the functions of many other glands Extracts of the anterior lobe appear to contain a number of distinct active principles some of which involve the metabolism of the whole body resulting in extreme cases either in dwarfism or gigantism More specific effects are also produced on other glands such as the pancreas thyroid parathyroids and adrenals and the extracts contain three gonadotrophic substances Little is known concerning the chemistry of these substances all of which appear to be proteins

Of the various hormones associated with the anterior lobe most attention has been given to the gonadotrophic factors A product with high luteinising activity can be obtained from human pregnancy urine by precipitation with phosphomolybdic acid The precipitate is subsequently dissolved in ammonia and baryta is added After filtration the hormone is precipitated by the addition of alcohol and ether[34] Various other methods for the preparation of the hormone have also been reported[35 36] An alternative source of the follicle stimulating hormone is the serum of pregnant mares from which a highly active preparation can be obtained by removal of the inactive proteins with salicylsulphonic acid, followed by dialysis and fractional precipitation with acetone[37]

Adrenals

The adrenal glands consist of two parts the medulla and the cortex The medulla is the source of the hormones adrenaline and noradrenaline while the cortex provides a large number of steroid hormones (see page 77)

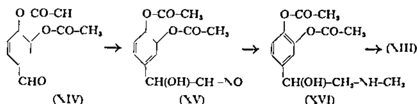
Adrenal Medulla In 1894 Oliver and Schafer[38] found that when an extract of the adrenal glands was injected intravenously into animals a marked rise in blood pressure resulted and in 1901 the active principle responsible for this action was isolated in crystalline form independently by Takamine[39] and by Aldrich[40] This active principle which was shown to be derived from the medulla and was given the name adrenaline was the first hormone to be isolated in a pure form

The hormone can be readily isolated in almost quantitative yield from the adrenal glands of oxen by utilising its basic properties The minced glands are extracted with hot dilute acid and after further heating the extract to coagulate most of the proteins it is filtered and concentrated under reduced pressure The concentrated residue is treated with two or three times its volume of alcohol and after filtration

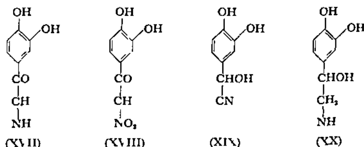
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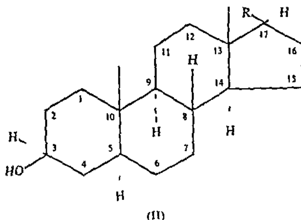
racemised by heating with hydrochloric acid and further resolution as before gives more (—) adrenaline This process may be repeated until practically the whole of the synthetic product has been converted into the physiologically more active lævo form identical with the compound isolated from the medulla In an alternative synthesis due to Nagai[45] nitromethane is condensed with the diacetyl derivative of proto catechuic aldehyde (XIV) and the reduction of the product (XV) with zinc dust and acetic acid in the presence of formaldehyde gives the diacetyl derivative of adrenaline (XVI) which may then be subjected to acid hydrolysis to give adrenaline (XIII)



Noradrenaline ($C_8H_{11}O_3N$) The presence in the medulla of the primary amine noradrenaline (XX) had long been suspected but it was not until 1949 that Holton[46] reported its presence in adrenal medullary tumours and its presence in the normal medulla was established shortly afterwards[47] It occurs as the (—) form Racemic noradrena line had been synthesised many years earlier by a variety of methods such as the reduction of either ω amino 3 4 dihydroxyacetophenone (XVII) ω nitro 3 4 dihydroxyacetophenone (XVIII) or the cyan hydrin of protocatechuic aldehyde (XIX)[48] and it had been introduced



under the name arterenol as a substitute for adrenaline but with little success Its resolution has recently been reported[49] Racemic noradrena line (XX) is a crystalline solid m p 191 with decomposition which is sparingly soluble in water ethyl alcohol and ether It is soluble in dilute acids and alkalis and yields a water soluble hydrochloride (m p 141) and oxalate (m p 175) (—) Noradrenaline melts at 216 5 to 218 corr ($[\alpha]_D^{25}$ —37 3) and its hydrochloride at 146° to 147 ($[\alpha]_D^{25}$ —39)



(II)

at positions 3 5 8 9 10 13 14 and 17 Such a structure will be capable of existence in 2^8 or 256 stereoisomeric forms Fortunately the main variations in stereochemical configuration encountered in natural products of the types under consideration are confined to the carbon atoms at positions 3 and 5 and the relative configurations of rings A and B The configurations between rings B and C and between rings C and D appear to be the same in nearly all steroids with the exception of the cardiac aglycones and therefore do not call for detailed treatment here The conventions usually adopted for the description and representation of substituted steroids have been expressed clearly by Shoppee[1] The configuration of substituents at the nuclear carbon atoms is represented by the suffix (α) or (β) according to whether the substituent lies below the plane of the paper or projects forward from the plane of the paper (as for example with the 3 hydroxyl group in cholesterol) Further whenever it is necessary to indicate a specific configuration (β) orientation is represented in formulæ by a full line bond whereas (α) orientation is represented by a broken line bond Thus in formula (II) the hydroxyl group at position 3 the methyl group at positions 10 and 13 the group R at position 17 and the hydrogen atom at position 8 are all (β) orientated and extend above the general plane of the ring system whereas the hydrogen atoms at positions 3 5 9 14 and 17 are all (α) orientated and lie below the plane of the ring system An excellent report of our knowledge of the stereochemical configuration of the steroid nucleus and of nuclear substituents has been published by Shoppee[1]

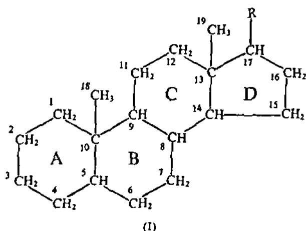
STEROLS

The sterols are constituents of animal and plant fats and oils They are neutral stable crystalline compounds which occur partly in the free state and partly esterified with higher fatty acids The usual method

CHAPTER IV

CHEMISTRY OF THE STEROID HORMONES

THE steroids are a group of compounds widely distributed in nature the structures of which are based on the reduced *cyclo*pentenophenanthrene system The parent system consists of four fused ring structures assembled as illustrated in formula (I) in wh ch the group R is a saturated or unsaturated hydrocarbon radical The group R is referred to as the side chain the remainder of the molecule being termed the *nucleus* *The four ring systems which are usually* though not always fully reduced are designated A B C and D and the carbon atoms are numbered as indicated The sex hormones and the hormones of the



(I)

adrenal cortex come within this group and among other types of *compounds which are included are the sterols* the bile acids the aglycones of the cardiac glycosides and the steroid sapogenins These various groups of compounds are thus closely related to the hormones and many of them are important as starting materials for the commercial preparation of the hormones They are also of value in the elucidation of the stereochemical configurations of the hormones

The stereochemistry of the steroids is a subject of considerable complexity which renders the total synthesis of any single member of this class a matter of great practical difficulty If for purposes of illustration *the fully saturated structure (II) is considered* it will be observed that the molecule contains eight asymmetric carbon atoms namely those

(IV)

in ring A This is a characteristic transformation associated with the oxidation of all 3 hydroxy steroids which contain a double bond between positions 5 and 6

Sitosterol ($C_{29}H_{50}O$) (V) the main sterol of plants is known in three forms termed α β and γ sitosterol The main constituent β sitosterol contains a secondary alcohol group at position 3 and an ethenoid link between positions 5 and 6 On oxidation it gives β sitostenone which has a ketonic group at position 3 and in which the double bond now occupies the position between the carbon atoms at positions 4 and 5 It differs from cholesterol in the side chain attached to position 17 which now consists of a saturated *iso*decyl group $C_{10}H_{21}$ Hydrogenation of β sitosterol gives stigmastanol (sitostanol) $C_{29}H_{52}O$

(V)

Stigmasterol ($C_{29}H_{48}O$) (VI) is found with β sitosterol in soya and calabar beans It differs from β sitosterol only in having a double bond in the hydrocarbon side chain and on hydrogenation it takes up two molecular proportions of hydrogen to give stigmastanol identical with that obtained by the hydrogenation of β sitosterol

(VI)

Ergosterol ($C_{28}H_{44}O$) (VII) is the main sterold constituent of yeast It mainly differs from cholesterol in having three double bonds instead of one One of these additional double bonds is situated between the carbon atoms at positions 7 and 8 and the other resides in the hydrocarbon side chain attached to the carbon atom at position 17 which contains nine carbon atoms Three molecular proportions of hydrogen are absorbed on catalytic

(VII)

of isolation consists in subjecting a neutral extract to hydrolysis with alcoholic sodium or potassium hydroxide and extracting the unsaponifiable matter with ether or light petroleum. In many cases the sterols isolated from natural sources consist of mixtures of closely related compounds. All sterols contain a (β) orientated hydroxyl group attached *to position 3 in the reduced cyclopentenophenanthrene* system. Sterols obtained from animal sources are termed zoosterols and those from plants phytosterols. The more important sterols are listed in Table VI.

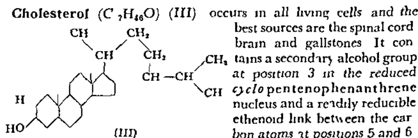
TABLE VI

STEROLS

Name	*Formula*	*Number of Double Bonds*	*M p*	$[\alpha]_D$	*Occurrence*
Cholesterol	$C_{27}H_{46}O$	1	150	−38.8	Animal cells
Ergosterol	$C_{28}H_{44}O$	3	163	−133	Yeast, Ergot
β-Sitosterol	$C_{29}H_{50}O$	1	146	−42.4	Plants
Stigmasterol	$C_{29}H_{48}O$	2	170	−45	Soya bean
Zymosterol	$C_{27}H_{44}O$	2	110	+47.3	Yeast
Fucosterol	$C_{29}H_{48}O$	2	124	−38.4	Algæ
Coprosterol (Coprostanol)	$C_{27}H_{48}O$	*0*	*102*	+23.5	*Fæces*

In these compounds the group R of formulæ (I) and (II) consists of a hydrocarbon chain containing from eight to ten carbon atoms, and where is coprosterol (coprostanol) is fully saturated the other members contain from one to three double bonds. Cholesterol, β-sitosterol and stigmasterol have been used as starting materials for the synthesis of sex hormones.

Cholesterol ($C_{27}H_{46}O$) *(III)* occurs in all living cells and the best sources are the spinal cord, brain and gallstones. It contains a secondary alcohol group *at position 3 in the reduced cyclo*pentenophenanthrene nucleus and a readily reducible ethenoid link between the car*bon atoms at positions 5 and 6*.



The side chain attached to the carbon atom at position 17 is a saturated *iso* octyl group C_8H_{17}. Oxidation of cholesterol gives the 3-keto steroid known as cholestenone (IV), in which the double bond has moved from between positions *5 and 6 in ring* B *to* between positions 4 and 5

(IX) (X)

Further in the oxidation of cholesteryl acetate dibromide (XI) by means of chromic acid 3(β) acetoxy Δ^5 cholenic acid (XII) is formed as a by product and this acid can be degraded to 3(β) acetoxy Δ^5 bisnorcholenic acid (X) by means of the Wieland Barbier process

(XI) (XII)

In this method an ester (XIII) of 3(β) acetoxy Δ^5-cholenic acid is treated with a Grignard reagent such as phenylmagnesium bromide and the resulting tertiary alcohol is dehydrated and oxidised to give 3(β) acetoxy Δ^5 norcholenic acid (XIV) as indicated below

(XIII)

(XIV)

This sequence of reactions is then repeated on the ethyl ester of (XIV) which leads in turn to the production of 3(β) acetoxy Δ^5 bisnorcholenic

hydrogenation giving ergostanol and oxidation of ergostanyl acetate gives 3(β) acetoxy nor 5 *allocholanic acid (see below)*

Bile Acids

The bile acids are normal constituents of the secretions of the liver of man and animals Like the sterols the bile acids contain a hydroxyl group attached to the carbon atom at position 3 but this is now (α) orientated and in place of the hydrocarbon chain attached to the carbon atom at position 17 a shortened chain at this position now contains a carboxyl group which is responsible for the acidic properties of the molecule The basic substance of this class may be regarded as *lithocholic acid or 3(α) hydroxy* cholanic acid (VIII) The main members *of this series are given in Table* VII and each can be converted to the parent substance cholanic acid by oxidation of the hydroxyl group or groups to ketone groups followed by reduction by the Clemmensen method with amalgamated zinc and hydrochloric acid

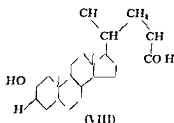


TABLE VII

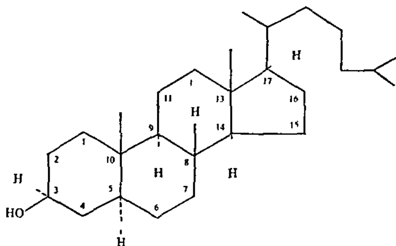
BILE ACIDS

Name	*Number of Hydroxyl Groups*	*Position of Hydroxyl Groups*	*Occurrence*
Lithocholic Acid	1	3	Cattle Bile
Cholic Acid	3	3 7 12	Human Bile
Deoxycholic Acid	2	3 12	Human Bile
Hyodeoxycholic Acid	2	3 6	Pig Bile

A number of unsaturated steroid acids are encountered in the processes used for the preparation of sex hormones from sterols Stigmasterol (VI) for example provides a suitable starting material for the *preparation of a steroid acid* since it contains a vulnerable double bond in the side chain which will be susceptible to oxidation Thus if the *hydroxyl group in stigmasterol is protected by acetylation and the* nuclear double bond by bromination as in stigmasteryl acetate dibromide (IX) *subsequent ozonolysis and debromination of the* product gives 3(β) acetoxy Δ^5 bisnorcholenic acid (X)

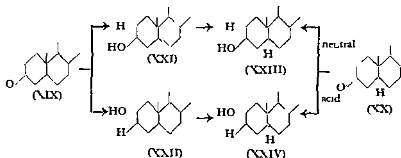
STEREOCHEMISTRY

Brief reference has already been made to the complexity of the stereochemistry of the sterols and related compounds In the molecule of cholestanol (XVIII) the rings A and B B and C and C and D are joined together in the *trans* configuration and the methyl groups or hydrogen atoms attached to the carbon atoms at positions 5 and 10 8 and 9 and 13 and 14 are also in the *trans* configuration



(XVIII)

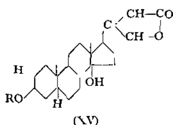
When Δ^5 cholestenone (XIX) is reduced to the corresponding alcohol by means of hydrogen in presence of Raney nickel catalyst the product is a mixture of cholesterol (XXI) and *epi*cholesterol (XXII) a new asymmetric centre having been introduced at the carbon atom at position 3 *Both sterols can be further reduced at the double bond by* means of hydrogen in the presence of a platinum catalyst to give cholestanol (XXIII) and *epi*cholestanol (XXIV) which can also be obtained directly from cholestanone (XX) by reduction under neutral and acid conditions respectively These changes may be represented as follows



acid (X) identical with the acid obtained from stigmasteryl acetate dibromide by ozonolysis

Aglycones of the Cardiac Glycosides

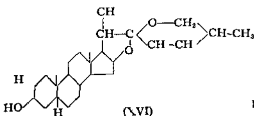
The digitalis glycosides occur in the leaves of varieties of digitalis (*Digitalis purpurea* *D lanata*) and closely related compounds are also found in other plants e g strophanthus They have a powerful action on the heart The glycosides isolated from *D purpurea* and *D lanata* are known as digitoxin (XV R=digitoxose residue) gitoxin and digoxin and can be hydrolysed to the aglycones digitoxigenin ($C_{23}H_{34}O_4$) (XV R=H) gitoxigenin ($C_{23}H_{34}O_5$) and digoxigenin ($C_{23}H_{34}O_5$) respectively together with the sugar digitoxose The sugar free compounds digitoxigenin gitoxigenin and digoxigenin are known to be lactones with a steroid nucleus largely as a result of the pioneer investigations of Jacobs and of Windaus and Stoll In digitoxigenin gitoxigenin and digoxigenin a secondary alcohol group occurs at position 3 in the reduced *cyclo*pentenophenanthrene system and the most characteristic feature of these compounds is the occurrence of an unsaturated lactone ring attached to the carbon atom at position 17 upon which the physiological activity depends



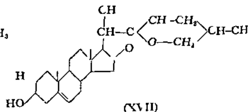
(XV)

Sapogenins

The saponins are a group of glycosides which occur widely in plants and the aglycones termed sapogenins derived from them belong to two groups Some give 3 methyl 1 2 *cyclo*pentenophenanthrene (Diel hydrocarbon) on dehydrogenation and are therefore closely related to the steroids whereas others give 1 2 7 trimethylnaphthalene (sapotalene) on dehydrogenation with selenium which indicates a close relationship to the triterpenes To the former class belong tigogenin ($C_{27}H_{44}O_3$) gitogenin ($C_{27}H_{44}O_4$) and digitogenin ($C_{27}H_{44}O_5$) A similar compound sarsasapogenin ($C_{27}H_{44}O_3$) (XVI) obtained from sarsaparilla (*Smilax* species) has been used for the synthesis of sex hormones Diosgenin ($C_{27}H_{42}O_3$) (XVII) isolated from the roots of *Trillium erectum* and from *Dioscorea tokoro* (Makino) has also been used for similar purposes

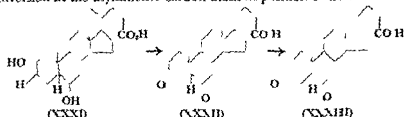


(XVI)



(XVII)

The significance of the stereochemical possibilities encountered in the steroids is perhaps best illustrated by reference to cholesterol and the bile acids which have been shown to belong to two different stereochemical series For example hyodeoxycholic acid from hog bile is a 3 6-dihydroxycholanic acid (XXXI) of the 5 *normal* series as also is the diketo acid (XXXII) obtained on oxidation but the latter compound is capable of rearrangement by a keto-enol change in the presence of acids or bases to a more stable stereoisomeride (XXXIII) of the 5 *allo* series which change must obviously be attributed to an inversion at the asymmetric carbon atom at position 5 thus



It was thus established that in the cholanic acids the rings A and B are in the *cis* configuration thus belonging to the *normal* or coprostane series whereas in the 5 *allo* acids the configuration is of the *trans* type encountered in derivatives of the cholestane series As previously stated the bile acids also differ from the sterols in having an (α) orientated hydroxyl group at position 3 To the former series belong also the hydrocarbons ætiocholane and pregnane while androstane and 5 *allo* pregnane belong to the latter

There is abundant evidence that œstrogenic androgenic progestational and cortical activity is highly sensitive to stereochemical structural changes Progesterone and deoxycorticosterone for example lose their activity in the corresponding 17 *iso*-compounds in which only the stereochemical configuration at the carbon atom at position 17 is changed In a similar manner *cis* testosterone and β œstradiol possess only a fraction of the activity associated with testosterone and α -œstradiol respectively

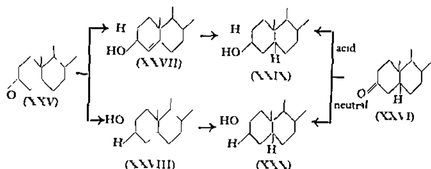
SEX HORMONES

The sex hormones are the natural secretions of the gonads or glands which are responsible for the sexual processes and for the secondary characteristic features which differentiate the male and female of a species These compounds are sometimes referred to as the secondary sex hormones since they are controlled by the protein like substances secreted into the blood from the anterior lobe of the pituitary which are sometimes called the primary sex hormones

The sex hormones are of three types

(a) Œstrogens (female or follicular hormones)
(b) Androgens (male hormones)
(c) Progesterone (the hormone of the corpus luteum)

In a similar manner Δ^4 cholestenone (XXV) may be reduced with aluminium *iso*propoxide to give a mixture of 3(β) hydroxy Δ^4 cholestene (*allo*cholesterol) (XXVII) and 3(α) hydroxy Δ^4 cholestene (*epi allo*cholesterol) (XXVIII) which on further catalytic reduction give coprosterol (coprostanol) (XXIX) and *epi*coprosterol (*epi*coprostanol) (XXX) respectively The latter are also obtained by reduction of coprostanone (XXVI) in acid and neutral media thus



Precipitation of a digitonide on the addition of a solution of digitonin in alcohol (90 per cent) provides a rough diagnostic test for 3(β) hydroxysteroids such as cholesterol cholestanol and coprosterol (coprostanol) in which the hydroxyl group is on the same side of the ring as the methyl group at position 10 *Epi*cholestanol and *epi*copro sterol (*epi*coprostanol) do not give such precipitates The configuration taken up by the 3 hydroxyl group in the reduction of cholestanone and coprostanone follows the Auwers Skita Rule which was deduced from a study of the reduction of the decalones and which states that under neutral conditions *trans* compounds are usually formed whereas reduc tion under acid conditions gives *cis* compounds Thus in cholestanol the hydroxyl group at position 3 and the hydrogen at position 5 are in the *trans* configuration since the cholestanol was obtained from choles tanone by catalytic reduction in neutral solution The above considera tions show that the isomerides cholestanol (or dihydrocholesterol) and coprosterol (coprostanol) differ only in the stereochemical configura tion at position 5 since the same isomeric relationship is maintained in the saturated hydrocarbons cholestane and coprostane In cholestane the configuration of rings A and B is of the *trans* decalin type whereas in coprostane the configuration is of the *cis* decalin type In cholestane the hydrogen atom at position 5 is in the *trans* position with reference to the methyl group at position 10 and this configuration gives rise to the 5 *allo* series *In coprostane the hydrogen atom at position* 5 and the methyl group at position 10 are in the *cis* configuration which gives rise to the 5 *normal* series The hydroxyl group at position 3 in cholesterol and in cholestanol is thus regarded as being in the *cis* position with reference to the methyl group at position 10

Little is known concerning the synthesis of the steroid hormones in the body. It is known that plant sterols derived from the diet do not provide a source for the cholesterol found in animals but are excreted in the fæces. Experiments with deuterium in place of hydrogen in water and in acetic acid fed to rats and mice have shown that cholesterol can be synthesised in the body from such simple molecules as those of water and acetic acid[2]. In similar experiments using deuterium as a tracer it has been shown that cholesterol can be converted into cholic acid in the dog[3] and into progesterone in the human female[4].

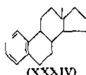
Œstrogens

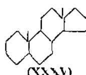
Isolation. Several methods have been described for the isolation of the œstrogenic hormones from pregnancy urine. In one method the urine is extracted with ether and the ethereal extract is evaporated. The residue is dissolved in methyl alcohol and the solution is shaken with light petroleum. The alcoholic solution is then diluted and extracted with ether, and the extract evaporated. The residue is treated with a mixture of alcohol (60 per cent.) and benzene, and whereas the alcoholic layer contains most of the œstriol, the benzene layer retains the œstrone. The crude hormones are hydrolysed with hydrochloric acid and taken up in ether. The ethereal solution is washed with aqueous sodium carbonate solution and extracted with aqueous sodium hydroxide solution, which removes the phenolic hormones and leaves the inactive pregnanediol. The hormones are precipitated from alkaline solution by the addition of acid and taken up in ether. The œstrone may be separated by high vacuum distillation, whereas the œstriol can be purified by precipitation from alcoholic solution by the addition of ether[5].

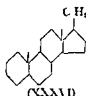
Improved methods of isolation have been reported by Marrian[6] in which the urine is first concentrated and then extracted at pH 2.5 to 3.0 with *n*-butyl alcohol after saturation with salt. The hormones are extracted as before with aqueous alkali and then purified. The œstriol is thus obtained as the sodium salt of the glucuronide. Girard and Sandulesco[7] utilised the Girard Reagent T (trimethylaminoacetohydrazide hydrochloride) for the isolation of œstrone, with which it forms a water-soluble derivative. Other workers have used the semicarbazone and the addition compound with quinoline for the isolation and purification of œstrone.

In isolating œstrone from the urine of pregnant mares it is necessary to make the urine strongly acid and the subsequent procedure varies from that recommended for human pregnancy urine. Doisy and his collaborators[8] employed a method in which the hormone is precipitated with benzoic acid. Beall and Marrian[9] used toluene for the initial extraction, and after various partitions between organic solvents and alkali the œstrone was precipitated as a mercury complex. The original literature should be consulted for details of these processes. A method

The œstrogens may be regarded as derivatives of œstrane (XXXIV) which contains eighteen carbon atoms and in which ring A contains three double bonds and is therefore aromatic in type. In a similar manner the androgens are based on the hydrocarbon androstane (XXXV) which contains nineteen carbon atoms and is fully saturated while progesterone is based on 5 *allo* pregnane (17[β] ethylandrostane) (XXXVI), a fully saturated hydrocarbon containing twenty one carbon atoms. The sex hormones and certain of their excretion products are given in Table VIII.


(XXXIV)


(XXXV)



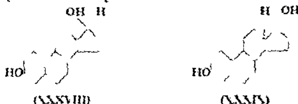
(XXXVI)

TABLE VIII
SEX HORMONES AND EXCRETION PRODUCTS

Name	*Formula*	*M p corr*	$[\alpha]_D$	*Occurrence*
Œstrogens				
(+) Œstrone	$C_{18}H_{22}O_2$	259	+170 in dioxan	Human pregnancy urine, urine of pregnant mares, bull's urine, stallion's urine, adrenal cortex
α Œstradiol	$C_{18}H_{24}O_2$	176 to 178	+81 in alcohol	Hog ovaries, urine of pregnant mares
Œstriol	$C_{18}H_{24}O_3$	281	+61 in alcohol	Human pregnancy urine
(+) Equilenin	$C_{18}H_{18}O_2$	258 to 259	+87 in dioxan	Urine of pregnant mares
Equilin	$C_{18}H_{20}O_2$	238 to 240	+308 in dioxan	Urine of pregnant mares
Androgens				
Androsterone	$C_{19}H_{30}O_2$	184 to 185	+94.6 in alcohol	Male urine
Dehydro*iso* androsterone	$C_{19}H_{28}O_2$	140 to 141; 152 to 153	+10.9 in alcohol	Male urine
Testosterone	$C_{19}H_{28}O_2$	155	+109 in alcohol	Testes
Progesterone	$C_{21}H_{30}O_2$	128, 121	+192	Sow ovaries, placenta, pregnancy urine, adrenal cortex

A total synthesis of œstrone has been reported by Anner and Miescher (see page 81)

α -Œstradiol ($C_{18}H_{24}O_2$) 3 17 Dihydroxy $\Delta^{1\,3\,5}$-œstratriene The reduction of œstrone to the secondary alcohol œstradiol was first reported by Schwenk and Hildebrandt in 1933[19] Their product was probably a mixture of the two isomerides now known as α (XXXVIII) and β -œstradiol (XXXIX) since reduction of the carbonyl group leads to the production of an additional asymmetric



carbon atom at position 17* The reduction product of œstrone was shown to be more potent in the Allen Doisy test than œstrone itself Of the two isomerides α -œstradiol was found to be about five times more active than (+)-œstrone whereas β -œstradiol is stated to be much less active Although α -œstradiol was first prepared synthetically by reduction of œstrone it was later isolated from the ovaries of sows by Doisy[20, 1] who believed it to be the active œstrogenic principle of the follicular fluid He regarded œstrone as a secondary product of less importance to the organism The two isomeric œstradiols each contain two hydroxyl groups one of which is phenolic in type and they give rise to a series of mono- and di-esters such as the 3-acetate (m p 136 5 to 137 5) 17 acetate (m p 215 to 217 5) and 3 17 diacetate (m p 127) Many of these esters possess a more persistent physiological action than free œstradiol and for this reason the monobenzoate which contains the benzoyloxy group attached to the aromatic ring and the dipropionate were introduced into clinical practice A method of preparing α -œstradiol from cholesterol has been reported by Inhoffen (see page 80)

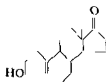
Œstriol ($C_{18}H_{24}O_3$) 3 16 17 Trihydroxy $\Delta^{1\,3\,5}$-œstratriene Shortly after the isolation of œstrone from human pregnancy urine by Doisy and by Butenandt the isolation from the same source of a second œstrogenic substance with a higher melting point was reported by Marrian[4, 22] This substance (XL) was at first regarded as a hydrate of the hydroxy ketone œstrone and this hypothesis was confirmed when Butenandt[5] showed that when the new substance was distilled in a high vacuum in the presence of potassium hydrogen sulphate œstrone was formed with elimination of water a conclusion which was later

* Compound (XXXVIII) called α -œstradiol by convention despite the fact that the hydroxyl group at position 17 has the (β)-orientation.

for the isolation of œstrone from the urine of stallions has been described by Cartland and his collaborators[10]

Whereas œstrone can be extracted completely with ether from a weakly alkaline solution much of the more acidic œstriol is not so extracted This difference in acidic strength permits a nearly quantitative separation[11] The hormones equilin and equilenin were isolated from the urine of pregnant mares largely by the use of the Girard Reagent T followed by distribution methods and crystallisations

Œstrone ($C_{18}H_{22}O_2$) (+) Œstrone 3 Hydroxy 17 keto-$\Delta^{1\,3\,5}$ œstratriene Ketohydroxyœstrin Œstrone (XXXVII) is a phenolic ketone It was isolated in a pure crystalline form in 1929 by Doisy Veler and Thayer[1] and independently by Butenandt[13] Early in the following year a similar substance was isolated by Dingemanse de Jongh Kober and Laqueur[14] In each instance the hormone was isolated from human pregnancy urine Œstrone has the normal chemical properties associated with a phenolic ketone and various derivatives have been prepared such as the acetate (m p 126) benzoate (m p 218 to 219) methyl ether (m p 168 5 to 169) oxime (m p 241 to 242) and semicarbazone (m p 266 to 267) As a phenol it couples with diazonium salts in alkaline solution In 1930 Zondek[15] showed that the urine of pregnant mares provided a richer source of œstrone than human pregnancy urine and this discovery led not only to the production of œstrone on a commercial basis with much improved methods of extraction and purification but also to the isolation of a number of closely related compounds such as equilin and (+) equilenin At a later date Zondek[16] showed that œstrone was also present in comparatively large quantities in the urine of stallions The œstrogenic hormone content of urine from various sources is shown in Table IX Œstrone has also been isolated from the urine of normal men[17] and the occurrence of œstrone in palm kernel oil has been reported by Butenandt and Jacobi[18]



(XXXVII)

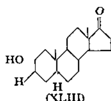
TABLE IX
ŒSTROGEN CONTENT OF URINE

Source of Urine	Œstrogen Content (mg per l)
Pregnant woman	2 1
Mature woman	0 042
Pregnant mare	10
Mature mare	0 020
Stallion	17
Bull	0 033

which acidic and phenolic compounds were removed by shaking with aqueous alkali Steam distillation of the neutral chloroform extract removed volatile impurities and after boiling successively with alkali and acid the unsaponifiable material containing the hormone was purified by partition between solvents firstly between benzene and light petroleum and then between light petroleum and alcohol (60 per cent) The crude androsterone was purified through its oxime[27] Subsequently improvements in this procedure were introduced involving the use of the Girard Reagent T and chromatography[28]

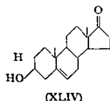
The isolation of dehydro*iso*androsterone from male urine has been described by Butenandt[29] Testosterone was isolated from testis tissue by David Dingemanse Freud and Laqueur[30] about 10 milligrams being obtained from 100 kilograms of tissue The original literature should be consulted for further details of these processes

Androsterone ($C_{19}H_{30}O_2$) 3(α) Hydroxy 17 keto-androstane Using the comb growth test as a guide Butenandt and Tscherning[27 31] were successful in isolating a pure crystalline male or androgenic hormone from human male urine This compound to which the name androsterone (XLIII) was given proved to be fully saturated and possessed a secondary alcohol group as well as a ketone group The analytical figures indicated that it is a steroid ketone bearing a close relationship to œstrone but without the acidic properties associated with a phenolic substance It gives typical derivatives such as the acetate (m p 164 5 to 165 5) the oxime (m p 209 to 211) and the semicarbazone (m p 276) Its structure was finally proved by a partial synthesis which was achieved by Ruzicka[3] in 1934 by the oxidation of *epi*cholestanyl acetate (see page 84) On oxidation androsterone gives androstandione which on reduction by Clemmensen s method with amalgamated zinc and hydrochloric acid gives the parent saturated hydrocarbon androstane

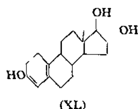


(XLIII)

Dehydro*iso*androsterone ($C_{19}H_{28}O_2$) 3(β) Hydroxy 17 keto Δ^5 androstene In 1934 Butenandt and Dannenbaum[33] isolated a second substance from human male urine which proved to be a physiologically inactive unsaturated chloro ketone formed during the process of isolation by the action of hydrochloric acid on the corresponding hydroxy ketone Treatment of the chloro compound with potassium benzoate followed by hydrolysis gave the hydroxy ketone which was also isolated from the urine in small yield and which was given the name dehydro*iso*androsterone (XLIV) Like androsterone it is a hydroxy ketone but with a



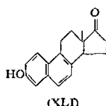
(XLIV)



(XL)

confirmed by Marrian and Haslewood[23] Cohen Marrian and Odell[24] showed that *œstriol was present in the pregnancy urine* in the form of a glycoside with glucuronic acid the glycosidic link involving the secondary hydroxyl group at position 16 in the *cyclo*pentane ring Œstriol has also been found in female willow catkins[25] This hormone is only about one fifth as active as œstrone and in con trast to œstrone it is able to penetrate the intestinal membranes and is *only slightly less active when administered by mouth than when* injected Œstriol gives rise to a series of esters and the tri acetate (m p 127) is said to be about ten times more active than the free triol Œstriol can be prepared from œstrone (see page 81)

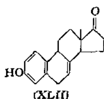
(+) Equilenin ($C_{18}H_{18}O$) 3 Hydroxy 17 keto-$\Delta^{1\ 3\ 5\ 6\ 8}$ œstra pentaene Using the newly discovered Girard Reagent T for the isolation and purification of the ketonic constituents of the urine of pregnant mares, Girard and his collaborators in 1932 and 1933[26] isolated two unsaturated ketones to which the names (+) equilenin and equilin were given Equilin contains two atoms of hydrogen less than œstrone and equilenin four atoms of hydrogen less (+) Equilenin



(XLI)

(XLI) has in fact the properties of a naphtha lene derivative since rings A and B are both aromatic in character It gives rise to esters and ethers such as the acetate (m p 156 to 157°) benzoate (m p 222 to 223) and methyl ether (m p 197 to 198) Its structure has been estab lished by degradation and by total synthesis (see page 82)

Equilin ($C_{18}H_{20}O_2$) 3 Hydroxy 17 keto $\Delta^{1\ 3\ 5\ 7}$ œstratetraene This hormone was isolated together with (+) equilenin by Girard and his collaborators in their work on the urine of pregnant mares Equilin (XLII) has not been synthesised but it can be converted by treat ment with palladium in nitrogen to (+) equi lenin It gives rise to esters and ethers such as the benzoate (m p 197 to 198) and the *methyl ether (m p 160 5 to 161 5)*



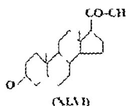
(XLII)

Androgens

Isolation Androsterone may be extracted from male urine by methods similar to those used in the extraction of œstrone from pregnancy urine but since androsterone is not phenolic in type it is found in the neutral portion of the extract In the earlier method of Butenandt the urine was made acid concentrated and extracted with chloroform after

cent) and extracting with small volumes of light petroleum to remove impurities After dilution the alcoholic solution is again extracted with light petroleum which removes the progesterone and leaves the œstrone in the aqueous alcohol Crystalline progesterone is obtained from the residue from the final light petroleum extract by trituration with ether in the cold The crude progesterone can be crystallised from dilute alcohol or pyridine

Progesterone ($C_{21}H_{30}O$) Δ^4 Pregnene 3 20 dione After the ripening and rupture of the follicle in the human ovary a yellow tissue known as the corpus luteum is formed and is responsible for the secretion of progesterone (XLVI) Butenandt[36 37 38] reported the isolation of the hormone in a pure crystalline form in 1934 and at about the same time similar reports were made independently by Slotta Ruschig and Fels[39] by Allen and Wintersteiner[40] and by Hartmann and Wettstein[41] although these early reports were somewhat confused owing to the existence of polymorphic forms melting at 121 and 128 respectively Progesterone is a diketone containing an $\alpha\beta$ double bond It has been synthesised by numerous methods from cholesterol and stigmasterol (see page 89)



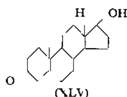
ADRENAL CORTICAL HORMONES

The hormones of the adrenal cortex are closely related chemically to the sex hormones Some twenty eight crystalline compounds of the steroid series have been isolated from extracts of the adrenal cortex

Isolation The separation of the medulla from the cortex is not practicable on a large scale The whole glands are therefore extracted with alcohol at room temperature and the alcohol is removed by distillation The residue is taken up in benzene and then in acetone and after partition between alcohol (70 per cent) and *n* hexane the alcohol layer is filtered through a column of Permutit to remove the adrenaline After evaporation the residue is taken up in water to eliminate resinous material After a further precipitation from alcoholic solution an active dry extract is obtained[4 43] In an alternative method the glands are extracted with acetone and after two partitions between alcohol (70 per cent) and light petroleum the material is extracted with ethylene dichloride[44] The active products are soluble both in water and in ether and benzene and the further purification of the hormones depends on repeated partition between solvents followed by the use of carbonyl reagents and chromatographic methods on both the free hormones and their acetates The original publications must be consulted for the detailed procedures employed Three groups of workers under Kendall Wintersteiner and Reichstein have thus isolated a large number of

3(β) hydroxyl group and it contains a double bond between the carbon atoms at positions 5 and 6 It gives rise to an acetate (m p 171 to 172) an oxime (m p 188 to 189) and a semicarbazone acetate (m p 268) On oxidation it gives an androstendione in which the double bond has migrated into ring A Its constitution was confirmed by its synthesis from cholesterol by oxidation of the acetate dibromide with chromic acid (see page 86)

Testosterone ($C_{19}H_{28}O_2$) 17 Hydroxy 3 keto Δ^4 androstene Physiological tests carried out on extracts from male urine indicated the presence of one or more androgenic compounds of greater potency than either androsterone or dehydro*iso*androsterone This activity was found to be unstable towards treatment with hot alkalis which *suggested the presence of an* αβ unsaturated ketone In 1935 David Dingemanse Freud and Laqueur[30] reported the isolation of the new hormone (XLV) to which the name testosterone was given It is an unsaturated hydroxy ketone with the same empirical formula as dehydro*iso*androsterone but whereas the latter compound had the secondary alcohol group at position 3 and the ketone group at position 17 in testosterone the positions are reversed Such a formulation is in agreement with its instability towards alkali which property is not shown by dehydro*iso*androsterone which is not an αβ unsaturated ketone The structure was confirmed by its synthesis from dehydro*iso*androsterone (see page 87) Testosterone gives rise to typical derivatives such as the acetate (m p 140 to 141) propionate (m p 121 to 123) benzoate (m p 198 to 200) and oxime (m p 222 to 223)

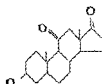


Progesterone

Isolation To isolate progesterone the ovaries of sows or the corpora lutea are extracted with alcohol and after removal of the solvent by distillation the residue is dissolved in ether The phospholipoids are precipitated with acetone cholesterol and fats are frozen out from solution in methyl alcohol and acidic compounds are removed with aqueous sodium bicarbonate solution Œstrone may be eliminated by partition between alcohol and light petroleum and the final purification is carried out by the use of a typical carbonyl reagent such as semicarbazide hydrochloride[34] The yield from 100 kilograms of ovaries is about 50 milligrams In an improved procedure due to Allen and Goetsch[35] the minced ovaries are extracted with methyl alcohol and diluted with an equal volume of water to precipitate *fatty substances* Subsequent extraction with light petroleum removes the progesterone which can be isolated in crude form on evaporation Further purification is effected by redissolving the crude extract in alcohol (70 per

acid of the acetate of 17 hydroxycorticosterone[51] Two partial syntheses from 3(α) acetoxy 11 ketobisnorcholanic acid and pregnane 3 21 diol 11 20 dione diacetate respectively have been reported (see page 97)

Adrenosterone ($C_{19}H_{24}O_3$) Δ^4 Androstene 3 11 17 trione This compound (LI) (m p 222 $[\alpha]_D$ + 262) was isolated by Reichstein[52] from the portions of the adrenal extract which were more soluble in ether It possesses no adrenocortical hormone activity but shows a definite androgenic action On hydrogenation it gives the saturated triketone which on reduction by the Clemmensen method gives the parent hydrocarbon androstane



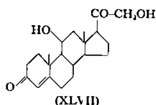
(LI)

Synthesis of the Steroid Hormones

The synthesis of the various steroid hormones constitutes a formidable problem because the reduced *cyclo*pentenophenanthrene system can exist in a large number of different stereoisomeric configurations and most synthetic processes will give rise to a complex mixture of stereo isomerides rather than to the one particular variety found in nature There is abundant evidence that in this series of compounds biological activity is intimately related to stereochemical configuration and it is therefore essential that any synthetic products prepared for use in medicine should possess the precise stereochemical structure found in the natural product In the testosterone and progesterone molecules there are eight asymmetric carbon atoms which implies the existence of a total of 256 stereoisomeric forms In the œstrone molecule which possesses one aromatic ring the number of asymmetric carbon atoms is reduced to four which implies only sixteen stereoisomeric configura tions whereas in the equilenin molecule with two aromatic rings there are only two asymmetric carbon atoms and four stereoisomeric forms It is therefore not surprising that of all the steroid hormones equilenin was the first to yield to a total synthesis and more recently this has been followed by a total synthesis of œstrone which also implies the total synthesis of œstradiol and œstriol No total synthesis has yet been reported of testosterone progesterone or any of the hormones of the adrenal cortex although recent developments in particular the syn thesis by Cornforth and Robinson[53] of a tricyclic ketone containing sixteen carbon atoms united in the precise stereochemical form found in coprostanone indicate that total syntheses of these and other similar hormones seem likely of achievement in the near future Numerous partial syntheses many of which have been developed into commercial processes have however been reported for dehydro*iso*androsterone

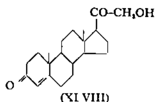
pure steroid compounds some of which are biologically active and others inactive The more important members are corticosterone deoxycortone dehydrocorticosterone cortisone, and adrenosterone Progesterone Δ^4 androstene 3 17 dione and œstrone have also been isolated by this method

Corticosterone ($C_{21}H_{30}O_4$) Δ^4 Pregnene 11 21 diol 3 20 dione Corticosterone (XLVII) (m p 180 to 182 $[\alpha]_D + 223$ in alcohol) has a constitution similar to that of progesterone but with hydroxyl groups attached to the carbon atoms at positions 11 and 21[45] The physiological activity is associated mainly with the hydroxyl group at position 21



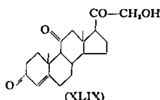
(XLVII)

Deoxycortone ($C_{21}H_{30}O_3$) Δ^4 Pregnen 21 ol 3 20 dione Deoxycorticosterone Desoxycorticosterone 21 Hydroxyprogesterone Deoxycortone (XLVIII) (m p 141 to 142 , $[\alpha]_D + 178$) differs from corticosterone by the absence of the hydroxyl group from position 11 It is more active than corticosterone and has been synthesised from dehydro*iso*androsterone and from 3 hydroxyætiocholenic acid (see page 95)



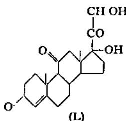
(XLVIII)

Dehydrocorticosterone ($C_{21}H_{28}O_4$) Δ^4 Pregnen 21 ol 3 11 20 trione This hormone (XLIX) (m p 174 to 181 5 $[\alpha]_D + 299$) was isolated by Mason Myers and Kendall[46] and it has also been prepared by Reichstein by the oxidation of corticosterone acetate followed by hydrolysis

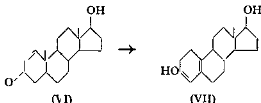


(XLIX)

Cortisone ($C_{21}H_{28}O_5$) Δ^4 Pregnen 17 21 diol 3 11 20 trione 17 Hydroxy 11 dehydrocorticosterone Kendall s Compound E This hormone (L) (m p 215 $[\alpha]_D + 209$) to which considerable attention has recently been drawn as a result of reports of its successful use in the treatment of rheumatic diseases has been isolated in four different laboratories[46 47 48 49 50] The hydroxyl group at position 17 has the (α) configuration The hormone has also been obtained by oxidation with chromic



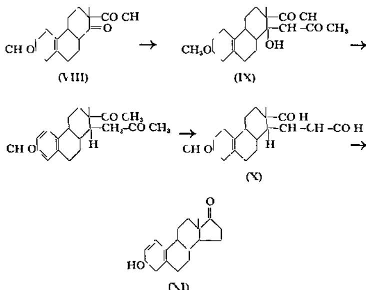
(L)



(VI) (VII)

from cholesterol have been investigated and confirmed by independent workers[57, 58] This conversion of an androgenic hormone to an œstro genic hormone proves that both types possess the same basic stereo chemical features

In the total synthesis of œstrone due to Anner and Miescher[59] one of the crystalline racemic modifications of the keto ester (VIII) previously prepared by Robinson and Walker[60] and by Bachmann Kushner and Stevenson[61] as a liquid mixture of racemates is subjected to a Refor matski reaction with methyl bromoacetate which gives four isomeric dicarboxylic esters (IX) which are then dehydrated and reduced By means of half hydrolysis and the applications of the Arndt Eistert homologation reaction the acid (X) is obtain which on cyclisation and decarboxylation gives racemic œstrone (XI) which is subsequently resolved to give (+) œstrone identical with the natural product



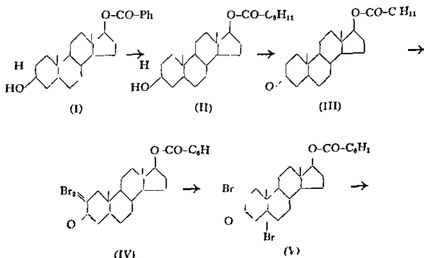
(VIII) (IX) (X) (XI)

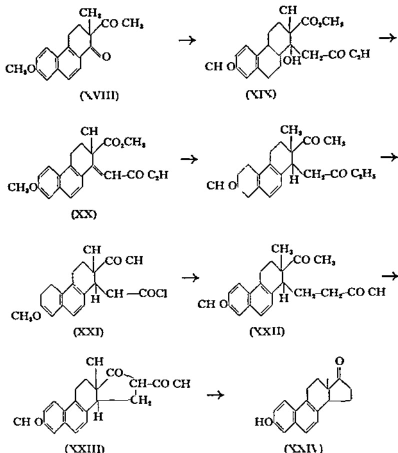
The conversion of œstrone to œstriol in an overall yield of 25 per cent has been reported[6] The methyl ether of œstrone (XII) is con verted by treatment with *iso*amyl nitrite in tertiary butyl alcohol into

testosterone progesterone and deoxycortone the processes use a sterol such as cholesterol or stigmasterol as the starting product By using such sterols in this way the correct stereochemical configuration for the final hormone is in the main already pre formed and the somewhat limited chemical changes involved in the synthesis can usually be made in such a way that the stereochemical configuration is controlled More recently it has been shown that certain sapogenins can be used as starting materials for the preparation of various steroid hormones

Œstrone Œstradiol and Œstriol Two methods are available for the synthesis of œstrone and hence of œstradiol and œstriol

In the Inhoffen[54, 55] process Δ^5 androstene $3(\beta)$ $17(\alpha)$ diol 17 benzoate (I) an intermediate obtained in the synthesis of testosterone from cholesterol is reduced catalytically to androstanediol 17 hexa hydrobenzoate (II), which on oxidation with chromic acid gives androstanolone 17 hexahydrobenzoate (III) Bromination in acetic acid solution gives 2 2 dibromoandrostanolone 17 hexahydrobenzoate (IV) which then undergoes rearrangement in the presence of hydrogen bromide in acetic acid to 2 4 dibromoandrostanolone 17 hexahydro benzoate (V) Removal of hydrogen bromide with collidine followed by hydrolysis gives the androstadienolone (VI) A solution of this compound in tetralin is then blown in a spray through a quartz tube maintained at 600 in an atmosphere of nitrogen and this results in the aromatisation of ring A and the expulsion of the angular methyl group The œstradiol (VII) thus formed is isolated in the form of a crystalline complex with tetralin from which the free œstradiol is obtained by distillation in steam[56] The essential stages in this synthesis of œstradiol

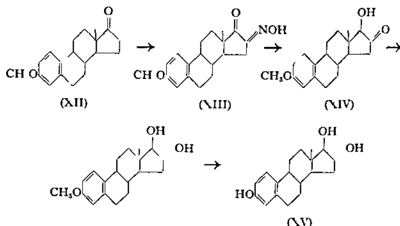




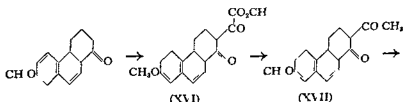
the half ester (XXI) and then the chain was lengthened by the Arndt Eistert procedure to give the ester (XXII) of the corresponding propionic acid cyclisation of which gave 16 carbomethoxy ± equilenin methyl ether (XXIII) The removal of the carbomethoxy group and the methyl group was effected by heating with acetic acid and hydrochloric acid and the resulting racemic equilenin (XXIV) was resolved by forming — menthoxyacetic esters In this manner (+) equilenin was obtained which was shown to be identical with the natural product together with (—)-equilenin Resolution of the product from the second racemic saturated acid gave (+) and (—) *iso*equilenin

In a more recent synthesis[64] 1 keto-7 methoxy 1 2 3 4-tetrahydrophenanthrene is converted by treatment with ethyl formate and sodium methoxide into (XXV) which with hydroxylamine gives the *iso*-oxazole (XXVI) This is converted by methylation into the cyanoketone (XXVII) which on condensation with methyl succinate in the

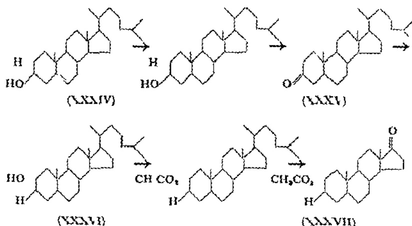
16 *iso* nitroso œstrone methyl ether (XIII) which on boiling with zinc dust in acetic acid solution gives 16 hydroxyœstrone methyl ether (XIV) Reduction with sodium and *iso*propyl alcohol converts the carbonyl group into a secondary alcohol group and subsequent demethylation with a mixture of hydrobromic acid and acetic acid gives œstriol (XV)



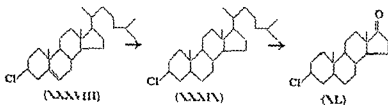
Equilenin Equilenin one of the female hormones found in the urine of pregnant mares was the first sex hormone to be synthesised completely This was achieved by Bachmann Cole and Wilds[63] in 1940 who obtained the four stereoisomerides one of which was shown to be identical with the natural product By the action of methyl oxalate on 7 methoxy 1 keto 1 2 3 4 tetrahydrophenanthrene they obtained the glyoxalate (XVI) which on heating lost carbon monoxide to give 7 methoxy 1 keto 2 carbomethoxy 1 2 3 4 tetrahydrophenanthrene (XVII) in good yield As a β ketonic ester this compound reacted with methyl iodide to give 7 methoxy 1 keto 2 methyl 2 carbomethoxy 1 2 3 4 tetrahydrophenanthrene (XVIII) This compound was then subjected to a Reformatski reaction with ethyl bromoacetate to give the hydroxy ester (XIX) which in turn was converted to a mixture of the two geometrically isomeric unsaturated esters (XX) Reduction of the double bond gave a mixture of the four possible isomeric saturated acids which formed two racemates which were separated by crystallisa tion Each racemic acid was in turn converted to the acid chloride of



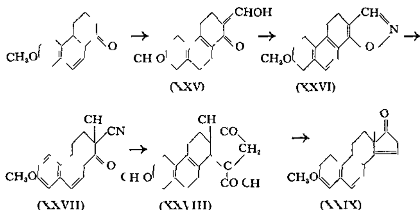
This method of synthesis indicates that androsterone belongs to the cholestane or *allo* series and is an alcohol of the *epi* type. The yields obtained in this oxidation are very small and Callow and Deanesly[65] using an improved process were able to obtain an overall yield of only 0.2 per cent. In this process cholesterol (XXXIV) is first reduced with hydrogen over a platinum catalyst to give dihydrocholesterol which on oxidation with chromic acid gives cholestanone (XXXV). Catalytic reduction of cholestanone with hydrogen in acid solution gives *epi*dihydrocholesterol (XXXVI) which is converted to the acetate and then subjected to oxidation with chromic acid under more drastic conditions. The androsterone acetate (XXXVII) thus formed is isolated from the mixture of oxidation products and unchanged *epi*dihydrocholesteryl acetate either by formation of the semicarbazone or with the use of a Girard reagent. The free androsterone (XXXIII) is then isolated by hydrolysis.



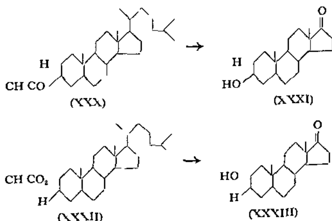
An improved synthesis of androsterone has been described by Marker[66, 67] who converted cholesterol (XXXIV) to cholesteryl chloride (XXXVIII) by treatment with phosphorus pentachloride and then reduced the chloride catalytically to give (α) cholestyl chloride (XXXIX), oxidation of which gave (α) chloroandrosterone (XL) which was hydrolysed to androsterone (XXXIII).

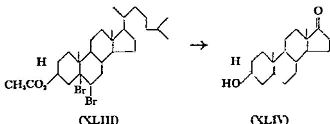


presence of potassium *tert* butoxide affords 15 carbomethoxy 14 15 dehydroequilenin methyl ether (XXVIII) Hydrolysis to the free acid followed by decarboxylation gives the methyl ether of 14 15 dehydro equilenin (XXIX) which on hydrogenation and demethylation gives racemic equilenin (XXIV) from which the natural hormone can be obtained by resolution as before



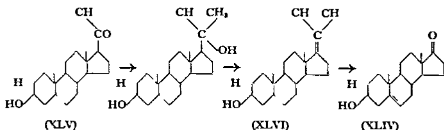
Androsterone Ruzicka showed that the oxidation of cholestanyl acetate (dihydrocholesteryl acetate) (XXX) with chromic anhydride in hot glacial acetic acid solution gave a small neutral fraction which proved to be 3 hydroxyætio*allo*cholan 17 one or *iso*androsterone (XXXI) a less active stereoisomer of the androsterone isolated from natural sources In order to obtain the correct stereochemical configuration Ruzicka[3] applied the same method of oxidation to *epi*dihydro cholesteryl acetate (XXXII) as well as to coprosteryl acetate and *epi*coprosteryl acetate The ketone (XXXIII) derived from *epi* dihydrocholesteryl acetate proved to be identical with androsterone





A similar method of synthesis was reported by Oppenauer[71] who used sitosterol obtained from soya beans in place of cholesterol and Butenandt Dannenbaum, Hanisch and Kudszus[29] described the preparation of dehydro*iso*androsterone from stigmasterol as well as from cholesterol In their preparation from cholesterol by the above method a yield of 2 8 per cent. was reported Full details of the process for the oxidation of cholesteryl acetate dibromide to dehydro*iso*androsterone acetate have been published[56]

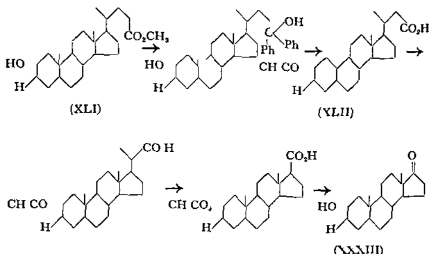
Using various sapogenins as starting materials Marker and his co-workers[7] have evolved a new method for the preparation of pregnenolone (XLV) which provides indirectly a new route to dehydro*iso*androsterone (XLIV) When treated with methylmagnesium halides pregnenolone gives a tertiary alcohol which loses water to give the *iso*propenyl derivative (XLVI) from which dehydro*iso*androsterone (XLIV) is obtained on ozonolysis



Testosterone The synthesis of testosterone was first accomplished almost simultaneously by Ruzicka[73 74] and by Butenandt[7] Dehydro*iso*androsterone (XLIV) obtained from cholesterol was reduced with sodium and propyl alcohol to androstene 3 17-diol (XLVII) and then converted to the di acetate (XLVIII) Partial hydrolysis of the di acetate gave 17 acetoxyandrosten 3-ol (XLIX) which was brominated at the double bond and oxidised to convert the hydroxyl group at position 3 to a ketone group Removal of the bromine atoms and hydrolysis then gave testosterone (Δ^4 androsten 17(β)-ol 3-one) (L) identical with the hormone isolated from male urine

Since cholesterol has the opposite configuration at position 3 to that present in androsterone a Walden inversion has taken place at one of the stages in this process

Androsterone has also been prepared by the degradation of a bile acid By the application of the Wieland Barbier degradation technique to an ester of 3 hydroxy*allo*cholanic acid (XLI) Dalmer von Werder Honigmann and Heyns[68] obtained androsterone (XXXIII) by the sequence of reactions illustrated below

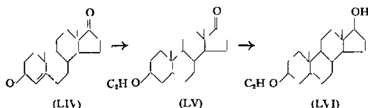


The 3 acetoxynor*allo*cholanic acid (XLII) was also obtained by the oxidation of the acetates of *epi*stigmastanol and *epi*sitostanol

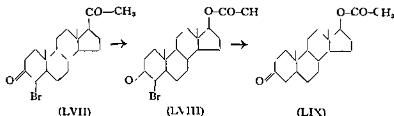
Dehydro*iso*androsterone This hormone was synthesised independently and almost simultaneously by Ruzicka and Wettstein[69] and by Wallis and Fernholz[70] by the oxidation of cholesteryl acetate dibromide (XLIII) with chromic acid In this oxidation the hydro carbon side chain attached to the carbon atom at position 17 is entirely removed leaving a ketone group in its place After debromination with zinc the ketone may be isolated as the semicarbazone but the yields originally obtained were of the order of about 1 per cent only Hydrolysis of the semicarbazone gave free dehydro*iso*androsterone (XLIV) identical with the compound previously isolated from male urine by Butenandt In addition to dehydro*iso*androsterone a number of by products are formed including Δ^5 pregnen 3(β) ol 20 one and Δ^5 3(β) hydroxyætiocholenic acid This method of synthesis from cholesterol established both the location of the double bond and the configuration of the hydroxyl group at position 3 which is therefore different from that present in the natural hormone androsterone

Later modifications were introduced whereby the reduction of dehydro*iso*androsterone acetate to 3 acetoxyandrosten 17 ol was effected catalytically with Raney nickel in methyl alcoholic solution and the oxidation of the androstendiol benzoate to testosterone ben zoate was carried out with aluminium *iso*propoxide and *cyclo*hexanone in the presence of toluene For clinical purposes the testosterone thus produced is converted into the propionate Full details of this process have been published[56] The overall yield of testosterone propionate from cholesterol is 2 4 per cent

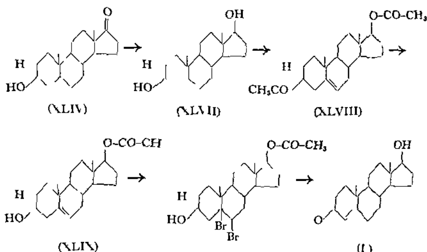
An alternative synthetic route to testosterone from dehydro*iso*andro sterone involves the oxidation of the latter to androstendione (LIV) which may then be converted into the 3 enol ethyl ether (LV) The ketone group at position 17 in the enol ether is reduced to the secondary alcohol to give a mixture of the 3 enol ethers of the two isomeric andro sten 17 ol 3 ones (LVI) which gives testosterone (L) and an isomeride on hydrolysis during which the free enol form reverts to the keto form[77]



A new method for the synthesis of testosterone using sapogenins as starting materials has been reported by Marker[78] Oxidation of pseudo sarsasapogenin leads to pregnane 3 20 dione and persulphate oxidation of 4 bromopregnane 3 20 dione (LVII) in acetic acid produces the 17 acetoxy compound (LVIII) which loses hydrogen bromide on treatment with pyridine to give testosterone acetate (LIX)

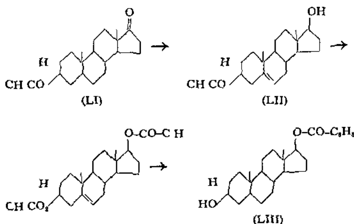


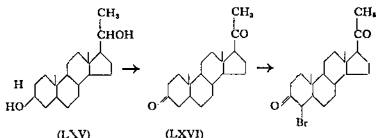
Progesterone Fernholz[79] showed in 1933 that stigmasteryl ace tate can be converted into the 5 6 dibromide (LX) which when sub mitted to ozonolysis and debromination gave Δ^5 3 acetoxybisnorcholenic acid (LXI) An ester of this acid was converted by Butenandt Westphal and Cobler[80] through the diphenyl carbinol into the unsaturated



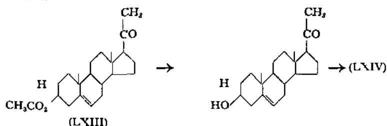
In the reduction of dehydro*iso*androsterone to androstendiol a new asymmetric centre has been created and the product will therefore consist of a mixture of two stereoisomeric androstendiols both of which are capable of conversion to the corresponding androsten 17 ol 3 one In the synthesis of testosterone it is therefore necessary to separate the correct stereochemical variety of androstendiol The second isomeride leads to the less active androsten 17(α) ol 3 one

An important improvement in the above synthesis was subsequently reported by Ruzicka Wettstein and Kagi[76] who reduced dehydro *iso*androsterone acetate (LI) to 3 acetoxyandrosten 17 ol (LII) and converted the latter to the 17 benzoate which mixed ester proved to be more suitable for partial hydrolysis than the 3 17 diacetate The resulting 17 benzoyloxyandrosten 3 ol (LIII) was then oxidised and debenzoylated to give testosterone (L)

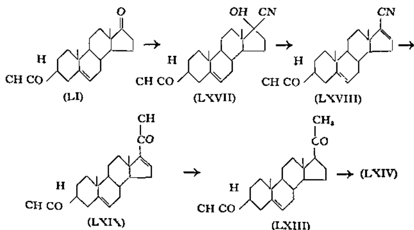




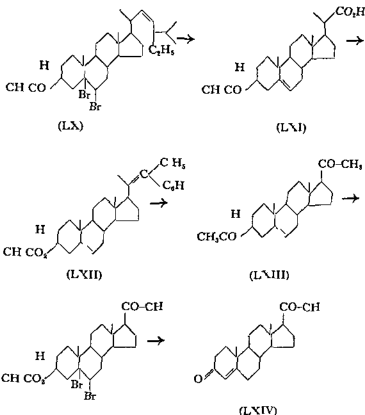
Progesterone may also be synthesised from Δ^5 pregnen 3(β) ol 20 one acetate (LXIII) a by product from the chromic acid oxidation of cholesteryl acetate dibromide to dehydro*iso*androsterone by hydrolysis and oxidation[84]



Butenandt[85] has described a synthesis of progesterone from dehydro *iso*androsterone acetate (LI) which on treatment with hydrogen cyanide gives the cyanhydrin (LXVII) which is then dehydrated to the nitrile (LXVIII) The unsaturated nitrile reacts with methylmagne sium bromide to give the ketone (LXIX) which is converted to pregnenolone acetate (LXIII) on reduction The pregnenolone acetate obtained in this way is converted into progesterone (LXIV) as des cribed above

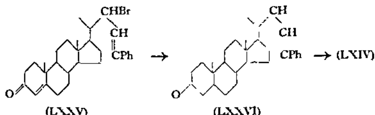


compound (LXII) Protection of the double bond between positions 5 and 6 by the addition of bromine followed by oxidation debromination and hydrolysis gave the acetate of Δ^5 pregnen 3(β) ol 20 one (LXIII) This compound after protection of the double bond by bromination was then oxidised and subsequent debromination gave progesterone (Δ^4 pregnen 3 20 dione) (LXIV) identical with the natural product



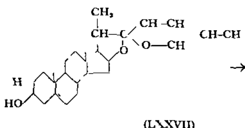
In the removal of the bromine with zinc dust the double bond is not regenerated at the original 5 6 position but appears at the 4 5 position in conjugation with the ketone group at the 3 position A similar synthesis was reported *independently by Fernholz*[81] *Details of this* process with many improvements have been published[56]

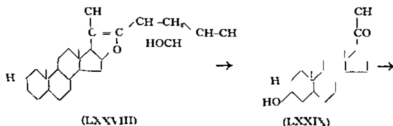
Butenandt[82] [83] *also prepared progesterone from pregnane* 3 20 diol (LXV) a reduction product of progesterone isolated from human *pregnancy* urine In one such method it was oxidised to pregnanedione (LXVI), which on bromination and subsequent treatment with pyridine to remove hydrogen bromide gave progesterone (LXIV)



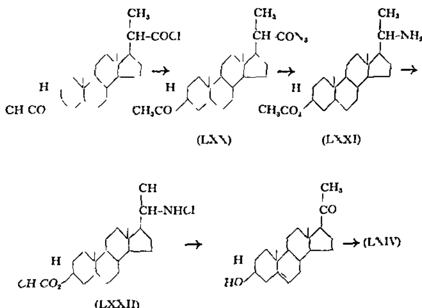
followed by treatment with N bromosuccinimide affords the bromo-compound (LXXV) which on loss of hydrogen bromide gives the ketone (LXXVI) This on oxidation yields progesterone (LXIV) The method provides an interesting example of the use of N bromosuccin imide which effects the removal of three carbon atoms in the side chain in one stage

An entirely new synthetic route to progesterone from certain sapo genins has been provided by the investigations of Marker[87] Diosgenin (LXXVII) obtained from trillin a glycoside in the root of *Trillium erectum* may be isomerised by heating with acetic anhydride at 200 to the pseudo form (LXXVIII) which on mild oxidation with chromic acid gives mainly $\Delta^{5\,16}$ pregnadien 3(β) ol 20 one (LXXIX) Hydrogenation of the latter over a palladium barium sulphate catalyst gives Δ^5 pregnen 3(β)-ol 20-one (LXXX) which can then be converted to progesterone (LXIV) by oxidation Alternative routes from diosgenin isolated from *Dioscorea tokoro* (Makino) have also been reported[88] Sarsasapogenin has similarly been used for a synthesis of pregnan 3 20 diol[89] (LXV)

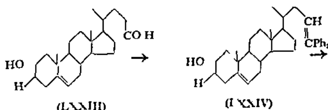




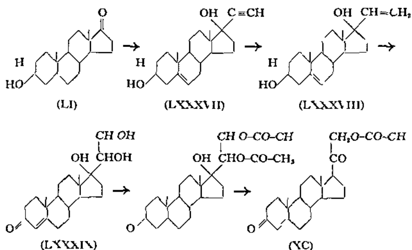
The degradation of Δ^5 3 acetoxybisnorcholenic acid to progesterone on a commercial scale was also achieved by Ruschig who converted the acid to the acid chloride and thence to the azide (LXX) The latter evolved nitrogen when heated and gave the *iso*cyanate which was converted to the amine (LXXI) on hydrolysis The amine was converted to the chloramine (LXXII) with hypochlorous acid and treatment with sodium ethoxide gave the ketimine, which was readily hydrolysed to pregnenolone This can be converted to progesterone (LXIV) by oxidation with aluminium *tert* butoxide in the presence of acetone and benzene[56]



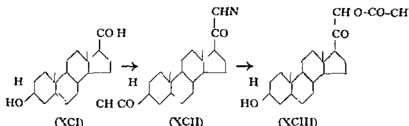
A number of new synthetic routes to progesterone have recently been reported by Meystre Wettstein and Miescher[86] In one of these Δ^5 3(α) hydroxycholenic acid (LXXIII) is converted through its methyl ester and reaction with phenyl magnesium bromide into the diphenylethylene (LXXIV) Oxidation of the hydroxyl group



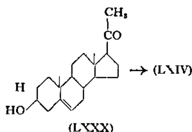
Deoxycortone The conversion of dehydro*iso*androsterone (LI) into deoxycortone was achieved in 1939 by Serini Logemann and Hildebrand[92] *who first prepared the addition compound with acety* lene (LXXXVII) namely ethinyl androsten 3 17 diol which was then partly reduced catalytically to the corresponding vinyl compound (LXXXVIII) After oxidation of the hydroxyl group at position 3 the vinyl group was converted to the glycol (LXXXIX) and then to the di acetate from which one molecular proportion of acetic acid was removed on heating with zinc dust to give deoxycortone acetate (XC) by molecular rearrangement Deoxycortone acetate can be



more conveniently prepared from 3 hydroxy Δ^5 ætiocholenic acid (XCI) a by product from the chromic acid oxidation of cholesteryl acetate dibromide which after acetylation is converted to the acid



chloride and then treated with diazomethane to give the diazo ketone (XCII) After hydrolysis and treatment with acetic acid nitrogen is evolved and the pregnendiolone acetate (XCIII) is formed which may then be oxidised by the Oppenauer method to give deoxycortone acetate[55, 93, 94] (XC)



(LXXX)

Corticosterone The partial synthesis of corticosterone has been reported by von Euw Lardon and Reichstein[90] The action of lead tetra acetate on pregnane 3 11 diol 20 one (LXXXI) gave pregnane 3 11 21 triol 20 one 21 acetate (LXXXII) which on oxidation with aluminium phenoxide in acetone afforded pregnane 11 21 diol 3 20 dione 21 acetate (LXXXIII) Bromination in acetic acid solution and subsequent dehydrobromination with pyridine yielded corticosterone acetate (LXXXIV) which was hydrolysed to give corticosterone (LXXXV) The pregnane 3 11 diol 20 one (LXXXI) was obtained from the methyl ester of the appropriate 3 11 dihydroxybisnorcholanic acid (LXXXVI) which in turn was obtained from bisnordeoxycholic acid[91]

CH_3 | CH–CO H — HO, H, HO → CH_3 | C=CPh — HO, H, HO → CH | CO — HO, H, HO →

(LXXXVI) (LXXXI)

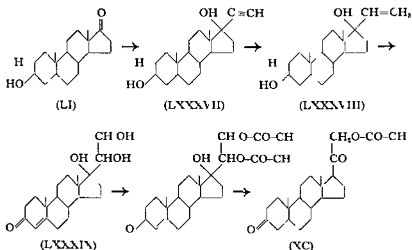
CH O-CO-CH_3 | CO — HO, H, HO → CH O-CO-CH_3 | CO — HO, O → CH O-CO-CH | CO — HO, O →

(LXXXII) (LXXXIII) (LXXXIV)

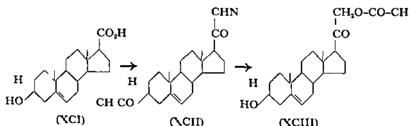
CH_2OH | CO — HO, O

(LXXXV)

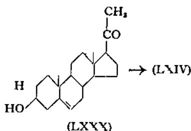
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(LXXXVI) (LXXXI)

(LXXXII) (LXXXIII) (LXXXIV)

(LXXXV)

HO COCH · CH-CO-O COCH · COCH

HO · O · O

(XCVI) → → (XCVII) →

Br COCH · COCH · COCl

HO · O · O

O · O · $CH CO_2$

→ (XCVIII) → (XCIX) →

CH O-CO-CH · CH OH

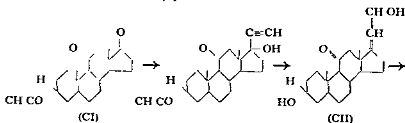
CO · CO

O · O

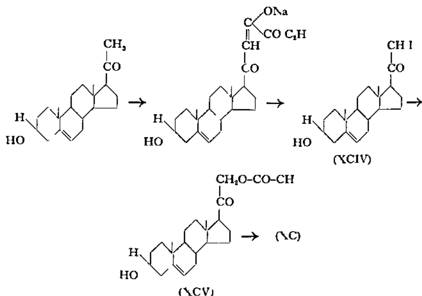
HO · O

→ (C)

Cortisone Two methods for the synthesis of this compound have been reported by Sarett In his first method[96] the diketone (CI) was condensed with acetylene and then subjected to partial reduction with a subsequent allylic rearrangement to give (CII) The 21 hydroxyl group was protected by succinoylation after which the hydroxyl group at position 3 was oxidised to give (CIII) which was treated with acetic anhydride and osmium tetroxide in order to hydroxylate the double bond The resulting compound (CIV) was treated successively with bromine and pyridine to introduce the double bond into ring A and the diacetate (CV) was hydrolysed and partially reacetylated to give the 21 monoacetate (CVI) which was then cautiously oxidised at the 20-hydroxyl group to give cortisone acetate (CVII) Adrenosterone was obtained as a by product



Deoxycortone acetate (XC) can also be obtained from pregnenolone obtained from 3 acetoxy Δ^5 bisnorcholenic acid by condensation with ethyl oxalate followed by hydrolysis of the resulting ester and treatment of the acid with iodine and alkali The iodo compound (XCIV) is then boiled with potassium acetate and acetone this gives 21 acetoxypregnenolone (XCV) which is oxidised with aluminium *tert* butoxide in the presence of *cyclo*hexanone and toluene to



deoxycortone acetate[56] (XC) Most of the other steroids isolated from the adrenal cortex contain either a keto or a hydroxyl group at position 11 and for these purposes cholesterol is not a suitable starting material Deoxycholic acid however has been successfully used as a starting material for the preparation of compounds for use in the synthesis of dehydrocorticosterone and cortisone

Dehydrocorticosterone In the synthesis of this substance the methyl ester of bisnordeoxycholic acid (XCVI) is oxidised and dehydrated by pyrolysis of the 12 benzoate to give the ester (XCVII) Addition of hypobromous acid at the double bond followed by oxidation and debromination gives the 3 11 diketo ester (XCVIII) The 3 keto group is reduced to the secondary alcohol the acetate of which is then converted to the acid chloride (XCIX) This is subsequently treated with diazomethane as in the synthesis of deoxycorticosterone from 3 hydroxyætiocholenic acid Finally after oxidation the double bond is introduced into ring A by bromination and subsequent elimination of hydrogen bromide with pyridine which gives dehydrocorticosterone (C) on hydrolysis[95]

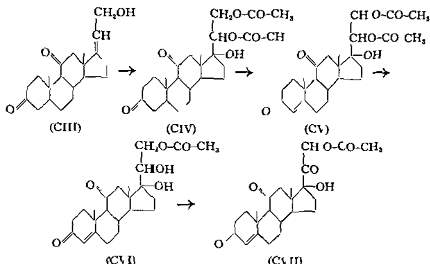
Improved methods of synthesis which avoid the use of the expensive osmium tetroxide have been outlined by Kendall[99] Sapogenins such as botogenin and the cardiac active principle sarmentogenin have also been suggested as raw materials for possible syntheses of cortisone Sarmentogenin obtained from the seeds of the tropical vine *Strophanthus sarmentosus* possesses the great advantage of having an hydroxyl group attached to position 11

Artificial Hormones

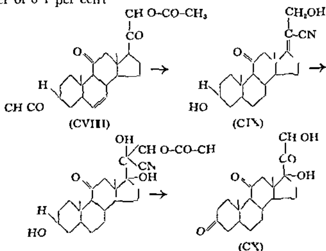
In the preceding pages it has been shown that most of the natural hormones have now been synthesised in the laboratory Many of these syntheses are in reality partial syntheses since they depend on a sterol bile acid or sapogenin as the starting material but in a few cases a total synthesis has been achieved as for example with equilenin and œstrone A large number of artificial hormones or compounds possessing hormone like action have been synthesised in the laboratory These belong mainly to the œstrogen series since the œstrogenic response is perhaps the least specific of all hormone reactions They have been referred to as synthetic œstrogens but artificial œstrogens is a more appropriate term These compounds are not found in nature and mostly they differ from the natural hormones in that they retain their activity when administered by mouth A comprehensive review of the chemistry of the artificial œstrogens and the relation between structure and activity has been published by Solmssen[100]

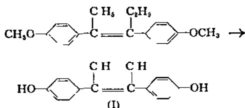
The fact that five different œstrogenic compounds had been isolated from natural sources namely (+)-œstrone α -œstradiol œstriol equilin and (+)-equilenin suggested that the physiological reactions associated with œstrus did not involve a high degree of specificity This observation led Dodds and his collaborators to look for synthetic compounds of simple structure which would be capable of reproducing all the known biological reactions of the natural œstrogenic hormones Among the earlier compounds investigated was 1 keto-1 2 3 4-tetrahydrophenanthrene which showed positive œstrogenic activity of a low order of potency Subsequently a large number of œstrogenically active substances were prepared some of which such as 9 10-dihydroxy 9 10 di *n* propyl 9 10 dihydro-1 2 5 6-dibenzanthracene were found to be almost equal to œstriol in activity[101] It was also demonstrated that the presence of the phenanthrene nucleus was not essential for œstrogenic activity and that a positive response was obtained even with such simple molecules as 4 4 dihydroxydiphenyl 4 4 dihydroxy diphenylmethane 4 4 -dihydroxydibenzyl and 4 4 -dihydroxystilbene[102]

Further elaboration of the stilbene molecule led to the preparation by Dodds Golberg Lawson and Robinson[103] of the highly active 4 4 dihydroxydiethylstilbene (I) generally known as stilbœstrol or diethylstilbœstrol This compound is more active than œstrone when

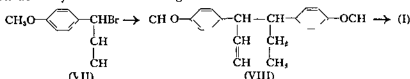


In the second synthesis[97, 98] pregnane 3 21 diol 11 20 dione diacetate (CVIII) was converted to the cyanhydrin and then dehydrated and hydrolysed to give (CIX) This was acetylated at the 21 position hydroxylated with osmium tetroxide followed by treatment with aqueous sodium sulphite oxidised at the 3 position and subsequently hydrolysed to give (CX) The 21 acetate was treated successively with bromine and boiling pyridine to introduce the double bond into ring A thus giving the acetate of cortisone (CVII) The yields obtained in the final stages were subsequently improved by employing the method of Mattox and Kendall[99] for the dehydrobromination stage In both methods of synthesis the ultimate starting material is deoxycholic acid Some thirty consecutive stages are involved and the overall yield is of the order of 0 1 per cent

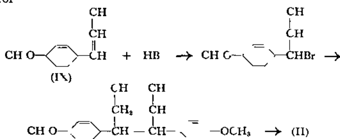




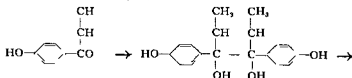
A more direct method of synthesis is due to Kharasch and Kleiman[105] who treated anethole hydrobromide (VII) with sodamide in liquid ammonia The resulting product regarded as (VIII) gave stilbœstrol on demethylation and rearrangement



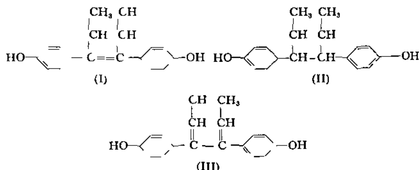
Hexœstrol (II) is prepared from anethole (IX) by addition of hydrogen bromide followed by a condensation of two molecules of the bromo compound in the presence of a metal such as sodium or magnesium The resulting dimethyl ether of hexœstrol is demethylated with alcoholic potassium hydroxide or with a solution of hydrobromic acid in glacial acetic acid The active product is the *meso* form of dihydro stilbœstrol[106 107 108]



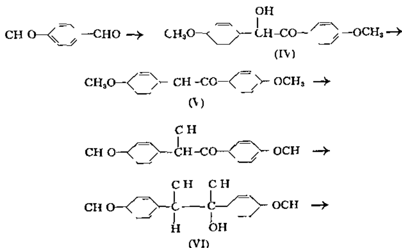
Dienœstrol (III) The preparation has been described by Dodds Golberg Lawson and Robinson[103] who reduced *p* hydroxypropio phenone with an aluminium amalgam to the pinacol (X) which on dehydration with acetic anhydride and acetyl chloride gave the diacetyl derivative of dienœstrol Hydrolysis with alcoholic potassium hydroxide gave dienœstrol (III)



administered subcutaneously and retains its activity when given by mouth In an attempt to effect a further simplification of the molecule by the elimination of one of the hydroxyphenyl groups in stilbœstrol anol (*p* hydroxypropenylbenzene) was investigated and found at first to be highly active but further investigation showed that this activity was due to an impurity which was later identified as 4 4 dihydroxy $\gamma\delta$ diphenyl *n* hexane (II) also known as hexœstrol which is a reduction product of stilbœstrol[104] Another compound of similar type with high oral activity is 4 4 dihydroxy $\gamma\delta$ diphenylhexadiene or dienœstrol (III)

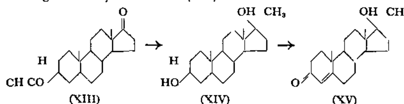


Stilbœstrol (I) is prepared from anisaldehyde which undergoes a benzoin type condensation to give anisoin (IV) Reduction with stannous chloride gives deoxyanisoin (V) which is treated successively with ethyl iodide and ethylmagnesium bromide The resulting tertiary alcohol (VI) is dehydrated to give the dimethyl ether of stilbœstrol which is demethylated with alcoholic potassium hydroxide[103] The active product obtained in this manner has the trans configuration

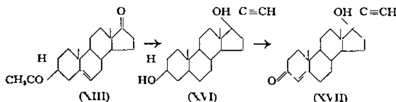


Steroid Derivatives Attempts have also been made to synthesise active compounds based on a steroid nucleus Among such compounds may be mentioned methyltestosterone ethisterone and ethinylœstradiol Methyltestosterone which is not found in nature possesses male hormone activity Ethisterone also is not a natural hormone but it shows the physiological reactions of progesterone Ethinylœstradiol is said to be a slightly more active œstrogen than α œstradiol Unlike the natural hormones all three compounds retain their activity when administered by mouth although at a somewhat lower level of activity

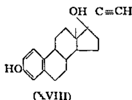
Methyltestosterone or 17 methyl Δ^4 androsten 17 ol 3 one (XV) is prepared from dehydro*iso*androsterone acetate (XIII) which on treatment with methyl magnesium bromide and subsequent hydrolysis gives 17 methylandrostene 3 17 diol (XIV) Oxidation of the latter with aluminium *iso*propoxide in the presence of *cyclo*hexanone and toluene gives methyltestosterone (XV)[119]

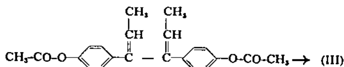


Ethisterone pregneninolone anhydrohydroxyprogesterone ethinyl testosterone or 17 ethinyl Δ^4 androsten 17 ol 3 one (XVII) is prepared from dehydro*iso*androsterone acetate (XIII) by treatment with potassium acetylide in liquid ammonia the resulting ethinyl androstendiol (XVI) being subjected to oxidation by the Oppenauer reaction[56 120 121 122]



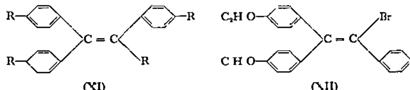
Ethinylœstradiol (XVIII) is obtained by the action of potassium acetylide on œstrone in liquid ammonia in much the same way that ethinyl androstendiol is obtained from dehydro*iso*androsterone[1 3]



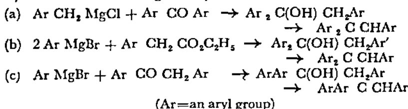


Subsequent improvements were introduced into this process by effecting the reduction to the pinacol with sodium amalgam or potassium amalgam in aqueous alkaline solution and by benzoylating the crude pinacol prior to dehydration and hydrolysis[109, 110] The chemistry of the artificial œstrogens of the stilbœstrol type has been reviewed by Jones[111]

Triphenylethylene Derivatives An entirely different group of artificial œstrogens is found in compounds of the triphenylethylene series Triphenylethylene (XI R=R′=H) was shown to be highly active by Dodds[11] and independently by Robson and Schonberg[113] and subsequently improved properties were claimed for triphenyl chloroethylene (XI R=H R′=Cl)[114], tri p anisylbromoethylene (XI, R=OMe R′=H)[115, 116] and β bromo β phenyl αα di (p ethoxy phenyl) ethylene (DBE) (XII)[117] Artificial œstrogens of this type are active by mouth and are characterised by having a prolonged action



The preparation of artificial œstrogens of the triphenylethylene series can be readily effected either (a) by the action of a benzyl magnesium halide on a benzophenone (b) by the action of a phenyl magnesium halide on an ester of phenylacetic acid or (c) by the action of a phenyl magnesium halide on a deoxybenzoin with subsequent dehydration of the resulting tertiary alcohol in each case thus



(a) Ar CH_2 MgCl + Ar CO Ar → Ar_2 C(OH) CH_2Ar → Ar_2 C CHAr

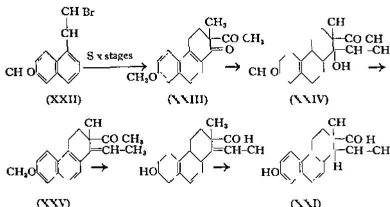
(b) 2 Ar MgBr + Ar CH_2 $CO_2C_2H_5$ → Ar_2 C(OH) CH_2Ar′ → Ar_2 C CHAr

(c) Ar MgBr + Ar CO CH_2 Ar → ArAr C(OH) CH_2Ar → ArAr C CHAr

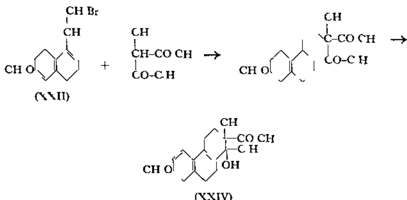
(Ar=an aryl group)

These reactions are capable of giving rise to a wide variety of substituted triphenylethylenes of various types and the corresponding chloro or bromo ethylenes may be obtained by the chlorination or bromination of either the carbinol or the ethylene[118] In the former case the reaction is accompanied by loss of water

Bisdehydrodoisynolic acid (XXI) and its methyl ether have been synthesised by Miescher[128] β (6 Methoxynaphthyl)ethyl bromide (XXII) was converted in six stages into the keto ester (XXIII) which was then treated with ethylmagnesium bromide This keto ester (XXIII) was also encountered in Bachmann s synthesis of equilenin[63] The resulting carbinol (XXIV) was dehydrated with formic acid to give the ester (XXV) which on hydrolysis and reduction gave racemic bisdehydrodoisynolic acid (XXI) This acid was subsequently resolved and the (—) acid was found to be identical with the acid prepared from equilenin



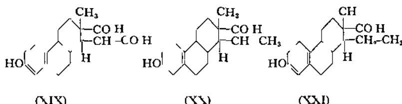
An alternative synthesis[129] utilises β (6 methoxynaphthyl)ethyl bromide (XXII) and methyl α propionylpropionate and proceeds by the following sequence of reactions giving the same carbinol (XXIV)



A detailed report on the stereochemistry and synthesis of these acids and of a large number of closely related compounds has been published by Shoppee[130]

Doisynolic Acid and Derivatives A number of highly active œstrogenic acids have been obtained by the partial degradation of natural œstrogens on fusion with potassium hydroxide The most active œstrogens at present known are found among these compounds the constitutions of which have been proved by total synthesis

In 1932 Marrian and Haslewood[1 4] isolated a dicarboxylic acid ($C_{18}H_2 O_5$) (XIX) since termed marrianolic acid by the fusion of œstriol with potassium hydroxide and shortly afterwards MacCorquodale Levin Thayer and Doisy[1 5] obtained a similar monocarboxylic acid ($C_{17}H_{2}O_3$) (XX) since termed doisynolic acid from similar treatment of (+) œstrone More recently this latter acid has been obtained by Heer and Miescher in 50 per cent yield by fusion of (+) œstrone or α œstradiol with potassium hydroxide at 275[126]



While marrianolic acid (XIX) is inactive doisynolic acid (XX) is practically as active as (+) œstrone by subcutaneous administration (—) Bisdehydrodoisynolic acid (XXI) obtained from (+) equilenin by fusion with potassium hydroxide[127] is even more potent being fully active in rats at a threshold dose of 0 05 microgram The figures in Table X indicate the relationship between the activity of these compounds the natural œstrogens and stilbœstrol

TABLE X

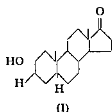
COMPARATIVE ACTIVITY OF SOME NATURAL AND ARTIFICIAL ŒSTROGENS

Compound	*M p*	[α] *in ethyl alcohol*	*Œstrus threshold value by s n le d se (microg ams)*	
			Subcutaneous	*O al*
Bisdehydrodoisynolic ac d (synthetic)	159 to 160	—116	0 05	0 05
(—) Bisdehydrodo synol c acid (from equ l nin)	161 to 162	—117	0 05	0 05
r Bisdehydrodo ynolic acid methyl ether	187	—	0 05	0 05
(+) Œstrone	259	+ 170 *	0 7	20–30
α Œstrad ol	176 to 178	+ 81	0 3–0 4	20–30
Stilbœstrol	168 to 171	—	0 3–0 4	0 7–1 0

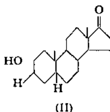
* in dioxan

excretion of both androgens and œstrogens and in particular of dehydro*iso*androsterone

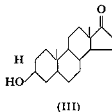
The main ketonic constituents of normal male and female urine are androsterone (I) 3 hydroxyætiocholan 17 one (II) and dehydro*iso* androsterone (III) These three compounds are 17 keto steroids and are interrelated stereochemically as shown in the following formulæ



(I)



(II)



(III)

Whereas androsterone and dehydro*iso*androsterone are androgenically active 3 hydroxyætiocholan 17 one has no such activity In order to estimate the total 17 keto steroid content of urine it is therefore not possible to use a method of bio assay but recourse has to be made to chemical and physical tests A colorimetric method introduced for this purpose by Zimmermann[135] and modified by Callow and Callow[136] depends on the production of a transient coloured complex with *m* dinitrobenzene in the presence of alkali By use of suitable colour filters this test can be made reasonably specific for 17 keto steroids More accurate determinations can be made by the polarographic analysis of a solution of the derivatives which the 17 keto steroids form with a Girard reagent The total 17 keto steroid fraction can be further classified into 3(α) and 3(β) hydroxy 17 keto steroid fractions by treatment with digitonin which will precipitate preferentially the dehydro*iso*androsterone (III) which alone of the three has the 3(β) configuration Improved methods for the determination of the qualitative and quantitative variations in the pattern of steroid excretion in human urine in health and disease have been reported[137] and the chemical characteristics of some thirty five (α) keto steroids and seven (β) keto steroids have been described[138]

In normal males the average output of neutral 17 keto steroids has been given as 14 milligrams a day whereas the average figure for normal females is 9 milligrams Removal of the ovaries does not appreciably affect the elimination of 17 keto steroids but in Addison s disease their elimination is markedly reduced which points to the adrenal cortex as the origin of these compounds in the female In the male they are considered to be derived in part from the adrenal cortex and in part from the testes Female patients with cancer of the adrenal cortex excrete excessive amounts of 17 keto steroids whereas male patients show an increased elimination of œstrogens

Excretion Products

The steroid hormones and their degradation products and derivatives are eliminated from the body mainly in the urine and there is much evidence to indicate that in many cases androgens and œstrogens are inactivated by combination with glucuronic acid or sulphuric acid Much valuable information has been obtained from qualitative and quantitative studies on the steroid content of human urine both during pregnancy and under normal and pathological conditions As a result of these investigations it has been possible to use the hormone content of urine (and of other body fluids) as a basis for various diagnostic tests of physiological and pathological conditions

The excretion of œstrone commences before puberty but after puberty there is a marked increase in œstrogenic substances in the urine which vary in quantity at different stages of the menstrual cycle During pregnancy the elimination of œstrogens increases steadily and both œstrone and œstriol are excreted in combination with glucuronic acid but after parturition the excretion decreases very rapidly and reaches normal levels within seven days Similar changes have been observed with the œstrogen content of fæces Appreciable quantities of œstrogens continue to be found in urine after the menopause An improved method for the determination of œstrogens in human pregnancy urine has been described by Stevenson and Marrian[131]

Progesterone seems to be rapidly metabolised in the body and much of it is excreted in the urine as pregnanediol glucuronide Pregnanediol is normally excreted only when the corpus luteum is actively producing progesterone and the quantity increases rapidly during pregnancy There is evidence however that the corpus luteum may not be the sole source of progesterone since Marker[13] has shown that pregnanediol is present in appreciable quantity in the urine of bulls A gradual fall in pregnanediol excretion during pregnancy indicates that the production of progesterone is inadequate and may result in abortion Estimations of pregnanediol in urine which can be carried out by the method of Venning[133] can also be of value in the early diagnosis of pregnancy An improved method of estimation has been reported by Huber[134]

The androgen content of urine increases gradually from early childhood both in males and females The excretion in adults varies within wide limits and women excrete about two thirds of the quantity excreted by men Both androsterone and dehydro*iso*androsterone have been isolated It is known that testosterone is rapidly metabolised in the body and part of it is excreted as androsterone

Numerous investigations have been carried out on the excretion of hormones in menstrual irregularities and abnormal conditions during pregnancy It has also been shown that changes in the activity of the adrenal cortex frequently result in excessive elimination of androgens and in patients with adrenal tumours there is a marked increase in the

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CHAPTER V

STANDARDISATION

DURING the past few decades there have been rapid developments in the technique of biological standardisation Researches into the vitamins and hormones have played an important part in stimulating these improvements the more so because of the need for accurate assessment of the potency of commercial preparations At first attempts were made to define potency in terms of animal units and there arose a welter of rat mouse and other units for various hormones indeed it still seems almost inevitable that the potency of new substances will continue to be defined in such terms despite their demonstrable inadequacy A rat or mouse unit of œstrogen for instance has been variously defined in different laboratories as the minimal amount which causes vaginal cornification in all of a group of animals as the amount causing it in the majority of a group or as the amount causing 50 per cent of the animals to react Quite apart from the vagueness of definition inherent in some of these requirements the variability of the same stock of animals from one time to another makes any such unit unstable Burn[1] for instance has shown how the frog unit for digitalis varied in the course of a year from 1310 to 2940 units per gramme and Emmens how the mouse unit for œstrone similarly varied from 0 64 to 1 50 international units

These difficulties are largely resolved by the recognition of the need for standard preparations with which any other substance of similar activity may be compared In theory so long as the mode of action of a sample of unknown potency is the same as that of the standard an estimate of the unknown potency derived from the simultaneous comparison of the actions of the standard and unknown should be independent of the test object used and of the particular reaction chosen as a response This situation is for the most part realised in practice although differences sometimes occur between estimates of potency when different species of animals or routes of administration are used These are often demonstrably due to the heterogeneity of one or both of the preparations being compared or to the presence of impurities in the unknown which affect its action differentially in various species of animal

International Standards The current international standard preparations designed for reference in the standardisation of hormones or hormone like substances are listed in Table XI

The preparations are kept at various centres under conditions designed to preserve their full potency and are made available to laboratories all over the world The distribution of standards in Great Britain is made from the National Institute for Medical Research in

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commercial scale in a similar state of purity Once the production of the hormones as pure chemical substances is achieved there is no object in continuing to use biological methods of estimation nor when it is achievable should manufacturers be content to produce impure products

Biological Assay

A satisfactory biological assay must fulfil certain specific requirements the relaxation of which should be permissible only in very special circumstances The minimal requirements are

(i) A standard reference preparation must be used simultaneously with the preparation under test

(ii) The assay must provide a valid unbiased estimate of the potency of the preparation under test *and* of the limits of error of this estimate at any required level of probability

(iii) The assay must provide evidence that the actions of the preparation under test and of the standard preparation do not differ

The first requirement guarantees that the comparison of standard and unknown shall be independent of secular variation in response

The second requirement concerns both the conduct of the test and its statistical evaluation It implies in general that the line relating the response to the dose or the logarithm of the dose (log dose) should be straight and that its slope should be determined on each occasion that an assay is performed that the variance of the response should in general be independent of the level of the response in assays based on graded responses and that the statistical methods by which the potency and its limits of error are determined should be mathematically sound It also implies that the animals or other test objects used in the assay are assigned although perhaps within certain permissible restrictions strictly at random to doses and substances

The third requirement is satisfied only when at least two different dosage groups are used for both the standard and the unknown When this is done the parallel nature of the two dose response lines can be examined and thus the similarity of action of the two substances is checked More than two dosage groups for each substance should be used unless there is already sufficient evidence that the dose response relationship is linear over the range encountered in the assay

Further characteristics of a satisfactory test method have been outlined by Gaddum[3] and by Bliss[4] These may be summarised as follows

(iv) The most accurate test method will be that for which the quantity s/b is minimal where s is the standard deviation of an individual result and b is the slope of the dose response line

(v) The living material receiving each dose of the standard and unknown must be as uniformly distributed among such dosage groups as is possible Potential sources of variation such as differences in response between litter mates sexes or strains of

London In order to conserve these preparations it is intended that laboratories should use them for the careful standardisation of their own local reference standards so that estimates of potency made by comparison with these local standards shall be referable with little error to the international standard preparation

TABLE XI

INTERNATIONAL STANDARD HORMONE PREPARATIONS

Preparation	*Date adopted*	*Unit (milligrams)*
Insulin (crude dry insulin hydrochloride)	1925	0 125
Insulin (pure crystalline insulin)	1935	0 0455
Pituitary (posterior lobe) powder	1925	0 5
Œstrone	1932	0 0001
Œstradiol monobenzoate	1935	0 0001
Androsterone	1935	0 1
Progesterone (corpus luteum hormone)	1935	1 0
Chorionic gonadotrophin	*1938*	*0 1*
Serum gonadotrophin	1938	0 25
Prolactin (galactin or mammotrophin)	1939	0 1

For each standard there is also an international unit This unit is the activity of a specified weight of each standard different for different standards and was usually defined for convenience with reference to the approximate quantity of standard required to elicit a response in a particular biological test which was popular at the time of determining the unit Since vaginal cornification in the mouse was and still is a method most frequently employed in the estimation of œstrogens the international unit for œstrone became the very small quantity 0 0001 milligram of the standard while the use of progestational changes in the rabbit uterus in the assay of progesterone led to the adoption of 1 0 milligram of this substance as a unit There is thus no general relationship between the weight of an international unit and the dosage required in medicine When an international standard exists however the potency of a preparation must be stated in terms of the number of units in one millilitre or gramme to which its action is equivalent The introduction of these standards has therefore meant that the potency of a preparation made anywhere can be accurately measured and compared with that of another without their physical proximity, and that the dosages of various drugs used in medicine can be so controlled that the safety of the patient and the reputation of the manufacturer are guarded alike

It should be noted that such standards are needed only when it is impossible or inconvenient to characterise a drug chemically Some existing standards in particular those relating to the sex hormones are rapidly falling into disuse since the standard preparations themselves are pure crystalline substances which can now be produced on a

The variance of a probit is not constant for different percentages and the dose response line has therefore to be fitted by using weights which are inversely proportional to the theoretical variance The line is fitted by a series of successive approximations and although only one or two cycles of calculation may be needed the arithmetic tends to become irksome even when equal numbers of test objects have been placed at each dosage level There are various approximate methods for estimating relative potency which often give a sufficiently good answer but none is adequate for the assessment of limits of error

Choosing a Test method Factors such as cost time and labour requirements will often strongly influence the choice of a particular test method for assaying preparations and it is rarely that a method combines optimal precision with desirable levels of these other factors High sensitivity is sometimes important as when insufficient quantities would be available for a more accurate but less sensitive method but it is not otherwise necessary in standardisation Specificity is of course of the highest importance A response which can be measured quantitatively is usually to be preferred since quantal responses are more troublesome statistically and usually give less accurate estimates for the same number of observations

A simple measure of response such as the weight of an organ is more likely to give a linear log dose response relationship than are less natural measures such as percentage increase in size or weight It is usually pointless to express the response in terms of some other variable such as body weight until the proper relationship has been established Thus although it might lead to a more accurate estimation of potency in a particular test if the weight of an organ be expressed as a percentage of the carcass weight instead of using the crude weight neither method is likely to give as accurate a result as the use of a correction factor derived by covariance analysis Similarly it seems to be widely assumed that when both initial and final measurements of the test object are possible in assays it is more accurate to use some such function as the difference between them as a criterion of response This may or may not be true frequently it is not Instead covariance analysis will enable a critical appraisal of the situation to be made

Computation The literature abounds with approximate and sometimes completely incorrect methods of computing relative potency and its errors Most of the approximate methods give no better an answer than can be guessed by anyone accustomed to dealing with assay data none of them gives a more accurate estimate of potency than a simple graphical fitting by a careful worker Moreover in actual practice some of them are more time consuming than the exact computation of potency from a balanced assay although it must be admitted that the computing of fiducial limits of error usually takes somewhat

animal must be so allocated to dosage groups that their influence can be isolated and examined in the subsequent statistical analysis Doses should not in general be adjusted for such factors as body weight by assuming some arbitrary and perhaps incorrect relationship but from the internal evidence of the test or group of tests of a like nature The statistical method appropriate to the making of such adjustments is covariance analysis an excellent example is given by Bliss and Marks[5] using the assay of insulin to exemplify the procedure

In hormone assays it is usually found that the logarithm of the dose is linearly related to the response Under these conditions there is rarely any point in failing to distribute test objects equally among the same number of dosage groups of both standard and unknown and to arrange doses so that successive dosage groups for each substance are spaced at the same equal logarithmic intervals Thus if sixty animals are available for a test of a particular substance against the standard in an assay in which it is deemed advisable to use three doses of each preparation each dose should be given to ten animals and doses should be in geometrical progression say 1 2 4 units of standard and 2 4 8 milligrams of unknown not in arithmetical progression say 1 2 3 units and 2 4, 6 milligrams When this scheme is followed computation is reduced to a minimum and the amount of information gained from the test is maximal

When several unknowns are simultaneously compared with the standard the rule requiring equal distribution of test objects between substances may be modified since the maximal amount of information about the potency of each unknown will on an average be obtained from an arrangement by which each dose of the standard is given to $\sqrt{N}$ times as many test objects as is each dose of each unknown where N is the number of unknowns It is not worth worrying about this in practice unless many unknowns are used at a time since the statistical computations are more laborious and the gain in information is not very great when N is small

Quantal Responses When all that is recorded about a group of test objects is whether or not each of its members exhibits some characteristic effect such as death or vaginal cornification the response is called quantal and special methods are needed in dealing with the data If the percentage response is plotted against either the dose or log dose the line relating the two is usually *S* shaped (sigmoid) To obtain a straight log dose response line it is necessary to convert the percentage of response to probits which are calculated on the assumption that the logarithms of the individual effective doses of the preparations under test are normally distributed[6, 7] Tables of probits corresponding to percentages of response from 0 1 to 99 9 are given by Bliss and in more detail by Fisher and Yates[8]

Limits of Error It is usual to accept as the limits of error of an assay a range which is estimated to include the true potency in either 95 or 99 per cent of cases When large numbers of test objects are used, this range is $M \pm 1\ 96\ s_M$ for the 95 per cent limits and $M \pm 2\ 58\ s_M$ for the 99 per cent limits With numbers below a hundred however and particularly with small total numbers like twenty or thirty animals in a test the distribution of t should be used as given in any modern textbook of statistics and the range $M \pm ts_M$ calculated Thus with a total of forty animals in a test, distributed between four groups t would be based on 36 degrees of freedom (or even less if such a factor as litter or strain difference is segregated in the test) the 95 per cent range would be $M \pm 2\ 03\ s_M$ and the 99 per cent range $M \pm 2\ 72\ s_M$ In quantal assays however the value of t remains 1 96 and 2 58 for the two probability levels whatever the number of animals employed

The error of any particular single assay cannot be predicted except within wide limits but the average error or the minimal expected error can be used for comparing the precision of different methods In the following pages it is imagined that a standard assay using a graded response is conducted with forty animals which will usually be distributed between four dosage groups two for each preparation When possible the estimated minimal error for P = 0 95 is calculated for each method of assay (P = 0 95 implies that the chance that the true value lies outside the range given is only 1 in 20) This error may be substantially exceeded in practice in particular if the mean responses to standard and unknown differ widely and if the value of the slope is not accurately known The minimal value expected for s_M in such a standard assay is $\frac{s^2}{10b^2}$ in a log dose response test supposing b to be determined with sufficient precision for the separate calculation of fiducial limits of error to be unnecessary This would often mean that an average value of b from previous assays must be available and be consistent with the new estimate of b provided by the assay being examined

The use of litter mates decreases error very considerably in all tests in which it has been investigated This decrease is of such magnitude that it is always worth the trouble of using litter mates when possible since the average test employing them has two to four times the accuracy (i e one half to one quarter of the variance) of a corresponding test with animals selected at random When possible it is similarly desirable to use cross over techniques in which the same animals are used at different times on the standard and unknown preparations in a test which is so balanced that overall differences in sensitivity between animals and differences due to secular variation are eliminated The elimination of differences between animals may reduce the error mean square to as little as one sixth of the value found without such a technique The errors quoted below are not calculated on a litter mate or cross over basis except where specifically stated

longer The use of balanced test methods of the type briefly described above permits factorial analysis of the assay, which is completed in a short time

Approximate methods are of most help when quantal responses are involved Here exact calculation tends to be more prolonged but the approximation is best confined to simple graphical estimation of potency No simple and sufficiently accurate approximate method is available for the estimation of limits of error, however and as these should be determined as a routine except in trivial circumstances the incidental determination of potency which their computation involves makes the approximate determination of potency superfluous The computational procedures for all types of assay discussed here are described by Emmens[9] These computations consist in essence of the determination of the following quantities when one unknown is compared with a standard

(i) The mean log dose of each preparation $\bar{X}$ and $\bar{Y}$ for standard and unknown respectively
(ii) The mean response to each preparation, $\bar{Y}_s$ and $\bar{Y}$
(iii) The common slope b
(iv) The standard deviation of a single observation s
(v) The standard error of the slope s_b

The logarithm of the ratio of the potency of the unknown to that of the standard M is given by

$$M = (\bar{Y} - \bar{X}) - \frac{(\bar{Y} - \bar{Y})}{b}$$

where $\bar{X}$ and $\bar{X}$ are measured in the same units such as milligrams The error of M is inherent only in the second term on the right hand side since there should be no significant error in administering doses If b is estimated with relatively high accuracy which in practice means that b/s_b is greater than about 8 then s_M the standard error of M is such that

$$s_M^2 = \frac{s^2}{b}\left(\frac{1}{n} + \frac{1}{n_u}\right) + \frac{(\bar{Y} - \bar{Y})\, s_b^2}{b^4}$$

where n and n are the numbers of observations on the standard and unknown respectively It will be seen that a minimal value for s_M occurs when $\bar{Y} = \bar{Y}$ and that the greater the difference in mean response the lower the accuracy of the assay

If b/s_b be less than about 8 this formula for s_M is not sufficiently accurate for the determination of limits of error and the so-called fiducial limits of error should be calculated by more exact means These are discussed by Irwin[10] The estimation of M however is not affected

laboratory and a dose response curve is given perhaps with standard errors of groups often for one preparation only Sometimes no actual assays have been conducted by a particular method and the reader is unable to decide whether even in the author s hands it gives reliable results In the absence of sound information as to their reliability it is therefore impossible to give more than passing mention or perhaps to do full justice to many suggested procedures

Anterior Lobe of the Pituitary

Thyrotrophic Hormone The setting up of an international standard for the thyrotrophic hormone was decided on in 1938[11] but has not yet been completed There is therefore no standard prepara tion or international unit in terms of which thyrotrophic extracts of the anterior lobe can be calibrated

There is only one method which can be particularly recommended for the assay of the thyrotrophic hormone that utilising the increase in weight of the thyroid gland of the guinea pig as described by Rowlands and Parkes[12] In guinea pigs injection of the thyrotrophic hormone causes hyperplasia of the thyroid gland (it does not do so in the rat which is therefore unsuitable for this type of test) and the weight of the stimulated gland is proportional to the logarithm of the dose over a wide range The method is described by Burn[1]

Rowlands and Parkes[12] used female guinea pigs approximately 200 grammes in weight but there seems to be no objection to using males instead The weight of the unstimulated gland appears to be linearly related to body weight and thus the use of animals of a relatively wide weight range might prove as accurate as long as the body weight is used as a concomitant variable in statistical analysis Subcutaneous injections are given for five days and the animals are killed on the sixth day The glands are fixed in Bouin s fluid overnight and weighed at the alcohol (70 per cent) stage in upgrading It is necessary to know the mean unstimulated weight of glands in the stock being used to ensure that a response is in fact being produced by the lowest doses employed

There is insufficient evidence upon which to base reliable estimates of the error of this test but it would appear that the minimal limits of error ($P = 0.95$) to be expected when two groups each of ten guinea pigs are used with each substance (forty animals all told) are about 77 to 129 per cent of the estimated potency

The thyroid gland of the chick also responds to the thyrotrophic hormone by an increase in weight and the same general test method may be employed using the chick thyroid as the test object as des cribed by Bergman and Turner[13] The authors state that this method is not so good as the guinea pig test but that it is more convenient when chicks are easily obtained However from the standard errors and slopes reported by Bergman and Turner it looks as if the chick test using day old White Leghorns is more accurate in their hands than

In quantal assays the standard test is increased to eighty animals because no worthwhile degree of precision can usually be expected with fewer test objects and the chance of obtaining 0 or 100 per cent of responses with only ten animals per group is too high for practical safety

In such assays it is also assumed that the average weight factor[4] is 0 5 This gives a slightly lower weight to a group than it might often have in practice and slightly wider limits of error than might be expected if all groups gave responses of between 20 and 80 per cent

METHODS OF ASSAYING RELATIVE POTENCY

The rest of this chapter is a survey of methods for standardising hormone preparations These cannot be given in complete detail and reference should be made to the original papers if it is desired to carry out an assay by a particular method or to descriptions given by Burn[1] and the British Pharmacopœia If Burn or the British Pharmacopœia has described a method in full reference is made to the fact It should be remembered however that many good assay methods have not been described by their authors in the light of modern statistics and that frequently the reader is advised to use a single group of test-objects and to refer to a standard curve given by the author of the method or to a similar curve which has been determined in his own laboratory or to conduct his assay by some other equally hazardous procedure Such recommendations represent a phase in the development of the subject which should by this time be disappearing It will therefore bear stressing again that satisfactory assays by whatever biological method the response is obtained supply internal evidence of their own reliability and that the primary basis of this is the simultaneous comparison *of standard and unknown preparations with at least two dosage groups* for each

In selecting test methods for particular recommendation it has also to be borne in mind that many methods recently developed for hormone assay have been devised primarily with the object of detecting small quantities in extracts of urine or tissues Thus sensitivity has been a first requirement and much ingenious work has been put into converting such sensitive tests of the presence of a hormone into satisfactory quantitative methods of assay Frequently however these methods are not as accurate as older techniques which use larger amounts of material and are therefore not to be preferred for the purposes of standardisation In general histological methods are not recommended because they are troublesome and usually incapable of satisfactory quantitative expression

There is a further difficulty which faces the reviewer of methods of assay Many suggested procedures look very promising some of them put forward as improvements in existing methods others quite new Too frequently however a method has been studied only in one

laboratory, and a dose response curve is given perhaps with standard errors of groups often for one preparation only Sometimes no actual assays have been conducted by a particular method and the reader is unable to decide whether even in the author s hands it gives reliable results In the absence of sound information as to their reliability it is therefore impossible to give more than passing mention or perhaps to do full justice to many suggested procedures

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the guinea pig test They also report a linear dose response relationship not a linear log dose response relationship Males are preferred to females because of a steeper slope

Lactogenic Hormone The international unit of the lactogenic hormone *(prolactin) is the specific activity of 0 1 milligram of the international standard preparation*

It is generally agreed that the growth of the crop glands of male or female pigeons provides a valid estimate of the lactogenic activity of preparations of the anterior lobe this was the only test recognised by the Third Conference on the Standardisation of Hormones held in Geneva in 1938[11] as giving sufficiently quantitative results for the comparison of preparations

The original method of Riddle Bates and Dykshorn[14] was to inject intramuscularly once daily for four days and to kill at ninety six hours The crop gland weight in milligrams was then multiplied by 150 and divided by the body weight and was found to be linearly related to $\log\left(\frac{\text{dose} \times 150}{\text{body weight}}\right)$ It is not very clear why this was done and later workers have successfully used simpler relationships Body weight has an influence on response and should be corrected for by covariance analysis When six daily injections were given and the birds were killed on the seventh day the mean slope relating crude crop gland weight in grammes to the logarithm of the dose for nine preparations was approximately 7 1[15] and the variance of the crop gland weight at the 4 gramme level of response was about 1 92 The minimal expected limits of error in a test using forty birds distributed equally between test and standard preparations is thus of the order of 75 to 133 per cent $(P = 0\ 95)$ and should be reduced if proper allowance be made for the correlation between body weight and response

Various modifications of the pigeon test involving the local application of the preparations under test have been tried as described for instance by Reece and Turner[16] Meites Bergman and Turner[17] and Hall[18] The general conclusion seems to be that although this type of test is much more sensitive it is not very consistent Hall however injects small doses locally over the crop sac either subcutaneously or intradermally using the left and right sides of the same bird for the standard and unknown and concludes that this modification gives reliable results Such a method could not readily be adapted for assaying preparations however unless a technique using the design known as symmetrical pairs were used as described by Bliss and Rose[19] When the figures submitted by the laboratories taking part in the examination of the contributions offered towards the international standard preparation were examined by Emmens[20] it was clear that the intradermal tests there reported were not as reliable as the more orthodox technique

Summaries of a variety of methods used in the assay of the lactogenic hormone are given in the Appendix to Emmens [0]

Growth Hormone No international standard exists for the estimation of the activity of growth promoting preparations of the anterior lobe of the pituitary gland

The estimation of growth promoting activity is usually made with rats either normal or hypophysectomised and the criterion of response may be an increase in body weight or various skeletal changes Marx Simpson and Evans[21] have made a careful comparison of the use of plateaued normal female rats as against hypophysectomised female rats for the growth test A plateaued rat is one which has reached a body weight of 220 to 280 grammes and is growing very slowly A rapid burst of growth may however be stimulated by the injection of the growth hormone Hypophysectomised rats are more sensitive to the growth hormone than plateaued animals but are more difficult to prepare and handle (see Burn[1])

A reasonably steep slope for the dose response curve is attained only after about fifteen days of injections which was adopted as a test period Marx *et al* [1] present their data in a form which makes statistical comparisons difficult but by reading back from their figures and tables it has been possible to estimate roughly the relative accuracy of the two methods The approximate slope of the line for plateaued rats is 27 and for hypophysectomised rats 18 when the weight increase in grammes is plotted against log dose The variances are about 47 and 43 respectively The expected minimal limits of error ($P = 0\ 95$) if forty rats are used in a balanced test are therefore 69 to 145 per cent for plateaued animals and 60 to 170 per cent for hypophysectomised animals The authors say that the accuracy is about the same for both types of test or even somewhat higher for normal rats They have tested many preparations and find a remarkably consistent value for the slope for plateaued rats which may therefore be recommended as suit able test objects Griffiths and Young[2] have described a different type of plateaued rat test Their rats received implantations of tablets of stilbœstrol a procedure which arrests growth and simulates hypophysectomy in many ways Such animals also respond to the action of the growth hormone but no critical evaluation of the accuracy of assays by this method is possible at present nor is it recommended by the authors as being particularly suitable

Evans Simpson Marx and Kibrick[23] have also elaborated a test using hypophysectomised rats in which the criterion of response is the width of uncalcified ephiphyseal cartilage in the tibia This test is stated to be three times as sensitive as the body weight method on a daily dose basis and since it takes only four days instead of fifteen it requires only about one tenth of the total dose From the figures in the text this method would appear to be quite accurate and the log dose response

line seems excellent giving a linear relationship over a range of from 5 to 200 micrograms of a local standard preparation The mean error of the test is stated to be 0 42 micrometer divisions but it is not clear whether this applies to means of groups or to individual observa tions Since the slope relating width of cartilage in scale divisions to log dose is 4 3 the minimal expected error of an assay is (P = 0 95) 86 to 116 per cent if twenty rats are used on the standard and twenty on the unknown and if 0 42 is in fact the standard deviation for individuals These limits seem very narrow for such a test, and it would appear that the error cited is that of means but since no details of the numbers involved are given it is not possible to estimate the probable errors of the test

Adrenocorticotrophic Hormone There is no international standard for the adrenocorticotrophic hormone Work on the standar disation of the hormone has been based on increases in adrenal weight in rats by Moon[24 25] or in chicks by Bates Riddle and Miller[26] No details of errors are given by Moon and the value of his tests cannot therefore be estimated Bates *et al* however present sufficient data for a rough estimate of the errors of the test to be made They injected two-day old White Leghorn cockerels subcutaneously three times a day for five days The dose response relationship was found to be linear but from the look of the figures the log dose response relationship also is probably linear over a wide enough range for assay purposes However, it is often more accurate to use a linear dose response relationship if this is possible The best fitting line for one series of chicks was adrenal weight in milligrams = 0 267 X + 10 1 where X was the dose of a particular preparation in milligrams The standard error of the mean of groups of ten chicks was said to be usually less than 0 7 milligram at low levels and rarely over 1 0 milligram at high levels of response If we take 0 7 as the average standard error to be expected using the dose response line above and distribute forty animals equally among five groups in a common zero 5 point assay as described by Finney[27] the expected limits of error (P = 0 95) will be approximately 80 to 120 per cent it being assumed that as wide a dosage range as is covered by the dose response line (up to 40 milligrams) is used In this type of assay a fifth group on zero dose is included in the calculations and the two dose response lines for standard and unknown are constrained to inter sect at zero dose

The adrenal weight test using hypophysectomised forty day male rats has been investigated by Simpson Evans and Li[8] It was treated by these authors as a test of the maintenance of adrenal weight in comparison with normal forty day controls but is adaptable as an assay in which the actual adrenal weight is used as the response The log dose response relationship appears linear when fifteen daily injec tions are given with a slope of about 9 5 when adrenal weight in

milligrams is plotted against the logarithm of the dose in milligrams The average variance was about 13 hence the minimal limits of error (P = 0 95) would appear to be in the neighbourhood of 57 to 174 per cent with the usual forty animal test used here as a basis for comparison

Sayers Sayers Tsan Ying and Long[29] have described a possible assay method using the fall in adrenal ascorbic acid and adrenal cholesterol which follows the administration of the adrenocorticotrophic hormone to both rat and guinea pig A similar study has been made by Reiss and Halkerston[30]

Gonadotrophins Methods for the assay of gonadotrophins of the anterior lobe are essentially similar to those described below for serum gonadotrophin and chorionic gonadotrophin As the content of follicle stimulating hormone and luteinising hormone varies from species to species it is usually necessary to use hypophysectomised test animals for differentiating between the two in a mixture No quantitative data *are available from which to recommend an assay procedure under such* circumstances nor does it seem to be in demand for the examination of commercial preparations Thus any assay of an anterior lobe preparation will as matters stand at present necessarily be approximate and the answer will vary according to the conditions of the test There is no international standard pituitary gonadotrophin

Chorionic Gonadotrophin

The international unit of chorionic gonadotrophin is the specific gonadotrophic activity of 0 1 milligram of the international standard preparation and it is to be used only for recording the activity of preparations of human pregnancy urine

There is a great variety of tests for gonadotrophic activity An opportunity to compare the efficiency of the more accurate methods occurred when the international standard was set up[31] because the various contributions offered towards the standard were assayed by thirty two different laboratories and many laboratories used several test methods However partly owing to the method of distribution of the contributions they were not all compared with each other by simultaneous assay and the errors of the assays examined are therefore not typical of an optimal procedure Nevertheless little doubt remained that tests employing vaginal cornification in the immature female rat or mouse ovarian weight in the rat and the formation of corpora lutea in the rat or mouse were the most accurate probably in the order given The vaginal cornification test and the ovarian weight test did not differ significantly in the magnitude of their error with the proviso that it was not always clear exactly how many animals had been employed in making the comparison in each case The numbers employed were however approximately equal

It has been decided to recommend the ovarian weight test using the immature female rat The reasons for this recommendation are

(i) It is more specific and does not demand control tests on spayed animals

(ii) It is a method which can also be recommended for the assay of serum gonadotrophin

(iii) When litter mates are used it is more accurate than any other test yet described

This method originally elaborated by Deanesly[3] is given in detail in the British Pharmacopœia It employs 40 to 80 gramme rats which are injected daily for five days and killed on the sixth day The ovaries are dissected fixed *in Bouin s fluid and weighed at the alcohol (70 per cent)* stage in upgrading Ovarian weight is linearly related to log dose and a maximal weight of about 40 milligrams is attained with chorionic gonadotrophin If litter mates are available these should be *distributed between all doses of standard and unknown The qualita* tive identity of action of the standard and unknown may be checked by histological examination of the ovaries the characteristic action of chorionic gonadotrophin is luteinisation The minimal limits of error *(P = 0 95) to be expected when litter mates are used and there are* forty rats in the test are 84 to 119 per cent When litter mates are not used, the limits (P = 0 95) are 70 to 140 per cent

The vaginal cornification method has the advantage that no dissec *tion is required and that the animals need not be killed Its disadvan* tages are the need for control tests to ensure the absence of œstrogenic material in the preparation under test and the fact that in the hands of some investigators it has often proved quite unreliable This it should be noted was not so in the many laboratories taking part in the assays mentioned above In these laboratories the slope of the probit log dose lines varied between 5 0 and 8 5 With the lower value of the slope, the limits of error (P = 0 95) of a test with twenty animals to a group would be at a minimum 75 to *134* per cent With the higher value the limits would be 84 to 119 per cent

Heard and Winton[33] used adult female rats which had been placed on a diet deficient in vitamins of the B group and had ceased to exhibit œstrus cycles *The slope for tests with these animals was 8 7 so that the* expected minimal limits of error for an assay with eighty such animals would also be about 84 to 119 per cent

It may be noted that the range of slopes reported for these vaginal cornification tests *is on the whole higher than those found when* vaginal cornification is used as the criterion of response in the assay of œstrogens (see below) This is quite feasible and would apparently imply that equal increments in log dose elicit more than equal incre ments in log œstrogen *production in the ovaries of the test animals*

Tests involving uterine weight the weights of male organs or vaginal opening in rodents ovulation in the rabbit and histological changes of

various types in these species are not in general to be recommended They are either inaccurate or inconvenient and often both Various test methods are however summarised in the Appendix to Emmens[31]

Serum Gonadotrophin

The international unit of serum gonadotrophin is the specific gonado trophic activity of 0 25 milligram of the international standard pre paration and it is to be used only for recording the activity of prepara tions of pregnant mares serum

The same tests may be used for comparing serum gonadotrophins as for comparing chorionic gonadotrophins When the assays of contribu tions towards the international standard preparation were examined[34] it was found that the method using ovarian weight in the rat was significantly more accurate than the general run of other tests There were insufficient data from which to judge the accuracy of the vaginal cornification test separately There was however no evidence that the various less accurate tests were giving biased estimates and it was concluded that independent of their accuracy they were measuring the same thing This means that since preparations of the serum of pregnant mares are known to exert both a follicle stimulating and a luteinising activity (although predominantly the former) the various contributions to the standard must be supposed to have contained the two principles in an approximately constant ratio if in fact there be two separate hormones

Under the stimulus of serum gonadotrophin the ovaries of the immature female rat may reach a weight of about 220 milligrams It is not however advisable to use the upper half of the dose response line because the variance is high and there is danger of rupturing large follicles when dissecting or handling the ovaries It is not necessary to give daily injections since a single subcutaneous dose of serum gonado trophin given six days prior to killing the animals is effective The qualitative identity of the standard and unknown may be checked by histological examination of the ovaries the characteristic action is stimulation of follicular growth In a forty rat test with litter mates the minimal limits of error ($P = 0\ 95$) to be expected are 93 to 108 per cent When litter mates are not used these limits of error are 84 to 119 per cent

Posterior Lobe of the Pituitary

The international unit of extracts of the posterior lobe is the specific activity (oxytocic antidiuretic or pressor) contained in 0 5 milligram of the standard preparation when extracted by the prescribed method which is given in the British Pharmacopœia

Oxytocic Activity The usual method of assay employs the uterus of the virgin guinea pig and is described in the British Pharmacopœia

It depends on the contractions of a uterine horn suspended in a bath containing Tyrode solution in response to the addition of an extract of the posterior lobe As it stands this assay is more an art than a science and cannot be assigned definite limits of error It would appear that so long as an experienced worker is satisfied with the way in which his particular uterine preparation is responding and so long as he makes valid comparisons of the type x y y x where x is a dose of the standard and y a dose of the unknown given in that temporal order the error of the test (P = 0 95) is usually about ± 15 *per cent* [35]

Hamburger[36] has described a method in which the average height of the contractions produced by several determinations at each of several dose levels of both standard and unknown is measured and the test is put on a more self contained and quantitative basis Hamburger *measured twenty to thirty contractions with each uterus ten to fifteen* on each preparation and constructed dose response curves for each These curves were either approximately linear or contained a sufficiently linear segment to allow of the application of the usual statistical methods for comparing two substances although in actual practice Hamburger made graphical comparisons Repeated determinations of the potency of three unknown dilutions of an extract of the posterior lobe were made by this method each with a different uterus The true potencies were 0 25 0 80 and 0 35 and the means of seven or eight tests were 0 24 0 77 and 0 31 respectively The percentage standard deviation of these three groups of tests was 13 5 10 6 and 10 0 The limits of error (P = 0 95) of a single test with one uterus are therefore approximately ± 20 per cent to ± 30 per cent but only when the technique of Hamburger is followed The usual technique would be expected to have a greater error than this because it employs less than twenty to thirty contractions in the total comparison and it is rather surprising that Gaddum[6] found a smaller error Bachinsky *et al* [37] measured the height of responses in a test in which each uterine horn was cut into four segments the eight pieces from the two horns being attached to eight separate levers With two doses of the standard and two of the test preparation the standard error of the estimate of potency was calculated to be less than 5 per cent in 18 out of 23 assays but in fact the true potency was known to be outside the calculated limits in ten instances Holton[38] used rat uteri in the more usual type of test giving doses each of standard and unknown and obtained standard errors of about 3 per cent but little is known about the differential actions of vasopressin and oxytocin in such a test (see also Burn[1])

Antidiuretic Activity Tests for estimating antidiuretic potency are described by Burn[1] and in the British Pharmacopœia Both use the rat and are based on the original work of Burn[39] The test depends on the comparison of the times elapsing until a maximum rate of excretion of urine is reached after the administration of water by stomach tube and

extract of the posterior lobe by injection Groups of four rats were used in a cross over technique the rats which had received the standard on one day receiving the unknown on the next day and *vice versa* Potency was calculated however with reference to a standard curve which was based on sixty four rats its position but not its slope being corrected in each test from the results obtained with the standard The curve looked as though a linear log dose response relationship held but no work based on this assumption is known It is not possible to estimate the error of the test Burn states that eight comparisons yielded results within 23 per cent of the known value the average error being 12 per cent Until the assay is put on a more satisfactory basis which allows of the computation of error from the internal evidence of the test or until many comparative assays are made by the present method this statement is the best that can be offered although much exploratory work has been undertaken Thus Ham and Landis[40] investigated various factors and concluded that under certain conditions the estimation of total chloride excretion is preferable to measurements of urine volume but did not use modern statistical methods in designing their procedure or evaluating their results

Pressor Activity The cat method for estimating pressor activity remains the only test which can be put forward at the present time The extract of the posterior lobe is injected into a vein of a decapitated cat but successive doses cannot be given frequently usually not more often than once every thirty minutes sometimes not more often than once an hour The rise in blood pressure which follows a constant dose decreases with successive injections at any rate if the injections are made more frequently than about once an hour This is an unsatisfactory test for only a few readings can be made with each cat and no statement is possible about limits of error The dog can be injected more frequently about once every fifteen minutes and Stewart[41] reports that it is always possible to distinguish differences of 20 per cent The rat behaves similarly and Landgrebe *et al* [42] may be consulted for the methods employed which appear to discriminate between 10 per cent dosage differences It would seem that rat methods are worth further investigation

Adrenals

Adrenal Cortical Hormones There are no international standards for the hormones of the adrenal cortex They fall into two main groups those which resemble deoxycortone and those which resemble corticosterone Of the first group deoxycortone appears to be the most potent substance for maintaining the life of adrenalectomised animals while of the second group 17 hydroxycorticosterone appears to be the most potent in affecting the metabolism of organic materials Clinical interest centres at present on deoxycortone which is best

assayed by its effect on the survival time or weight gain of adrenalectomised rats or the maintenance of normal conditions in adrenalectomised dogs No attempt will be made here to cover the field in its entirety since the subject is undergoing rapid development A detailed review by Thayer[43] has been published

Grollman[44] found that the survival of adrenalectomised animals is greatly influenced by the intake of sodium chloride and potassium ions He also found the gain in weight under standard dietary conditions to be a better criterion than survival time in rats but Thayer could not obtain accurate results by that method Thayer and his colleagues have not in fact found any test utilising survival weight gain sodium metabolism or renal function as measured by blood urea nitrogen which promises great accuracy Thus the characteristic actions of deoxycortone have not yet been made the basis of an assay method which can be assigned any but very approximate and wide limits of error Tests utilising a characteristic activity of corticosterone on the other hand attain considerable precision Unfortunately this activity the causing of an increase in glycogen or total fermentable sugar in the liver of adrenalectomised rats is not exerted by deoxycortone at all

In statistical comparison of the glycogen against total fermentable sugar test Eggleston Johnston and Dobriner[45] find that the latter is much more accurate They injected adrenalectomised rats hourly for 7 hours and killed at 7½ hours from the start of the test and then determined either the liver glycogen or total fermentable sugar The log dose response line for the latter was much the steeper each animal contributed about ten times as much information about relative potency as compared with those of the glycogen series In a test with twenty mice on the standard and 20 on the unknown the minimal limits of error ($P = 0\ 95$) to be expected in the two methods are

Glycogen	51 to 195 per cent
Total fermentable sugar	83 to 121 per cent

The error for the glycogen test would appear to be in line with that found by Dorfman Ross and Shipley[46] who used a similar technique with adrenalectomised mice and referred the results of series of tests with only one dosage level to a standard curve They found that when ten mice were used the error ($P = 0\ 95$) ranged from 50 to 250 per cent The predicted error was 41 to 250 per cent Their predicted error ($P = 0\ 95$) with forty mice per group (although all on one dose of the unknown) would thus appear to be about 60 to 160 per cent However a similar method employing the estimation of liver glycogen gives lower errors in the hands of Venning Kazmin and Bell[47] These investigators modified the Reinecke and Kendall[48] test and gave injections of the hormones in 5 per cent glucose obtaining parallel log dose response lines for various cortical steroids and minimal expected limits of error ($P = 0\ 95$) of about 80 to 125 per cent for the hypothetical forty mouse test used here as a standard of comparison

Adrenaline There is no international standard preparation of adrenaline The substance may be extracted from adrenal glands or synthesised and characterised as a pure compound For the purposes of standardisation a biological test is necessary only to ensure that a sample has the full activity of pure adrenaline when the methods described by Burn[1] may be used The blood pressure of the atropinised cat or dog gives very satisfactory estimates with standard errors of about 2 per cent[49] while the frog heart method[50] has about the same precision In this latter test winter male frogs were found to be preferable and assays of the x y y x type were used It is a simple method and appears to be very useful when frogs of the right type are available although no data were given as to the relative errors of other than winter male frogs It would seem to be unnecessary to attempt modifications of these tests which would give internal estimates of error so long as the laboratory performing them is regularly doing the same type of work and can keep a check on consistency The limits of error ($P = 0\ 95$) can apparently be kept within about 95 to 105 per cent if care be exercised and the identity of the substance concerned is not usually in question

Thyroid

There is no international standard for thyroxine or thyroid preparations The latter are usually assayed chemically on the basis that all the acid insoluble iodine is combined as thyroxine However the recent use of artificially iodinated proteins has led to a revival of interest in biological tests because not all the acid insoluble iodine in such proteins is contained in thyroxine or other substances of similar activity

Several methods for assaying thyroid preparations are given by Burn[1] none of them is very accurate and most are unwieldy More recently two promising new tests (described below) have been devised for which details of limits of error can be given The first one is an adaptation of the tadpole test first proposed by Lenhart[51] the second is a mammalian test so far investigated with albino mice

(i) *Xenopus Tadpole Method* This method was developed by Deanesly and Parkes[52] who describe methods for obtaining and rearing the tadpoles at any season of the year They decided to use eruption of the front legs as the response to thyroid activity Groups of five tadpoles 18 to 25 millimetres in length are placed in 200 millilitres of distilled water to which the substance to be assayed is added as a fine suspension The distilled water is changed to tap water after three days and the percentage of tadpoles showing eruption of one or both front legs is recorded on the seventh day The slopes for thyroxine and various iodinated proteins are substantially the same but are affected by temperature steeper slopes being obtained at 24 than at room

temperature At 24° the mean slope of nine tests was 5 95 With this *slope a test using four groups of twenty tadpoles* two groups on the standard and two on the unknown will have minimal limits of error (P = 0 95) of 78 to 128 per cent

The biological activity of eight preparations of iodinated plasma ardein or casein as measured by the tadpole test agreed very closely with the capacity of these preparations to stimulate milk yield in cows (Deanesly and Parkes[53]) However di iodotyrosine is active in mammals so that some slight doubt is cast on the specificity of the test This was one reason for the development of a new mammalian test

(ii) *Enclosed Vessel Technique with Mice* This method is described by Smith Emmens and Parkes[54] Young male albino mice are injected with the substances under test on days 1 3 and 5 and the test is performed two or three days after the last injection, that is on the seventh or eighth day Each mouse is placed in a separate 2 lb glass jar with an air tight lid the response is the survival time in minutes This is decreased by increasing the temperature and by injections of thyroxine or an iodinated protein The test is performed at 23 which gives survival times of convenient length and about a minimal variance The preliminary results with the test using male mice which are less variable than females indicate that with ten mice per group the minimal limits of error (P = 0 95) are about 73 to 137 per cent

The peculiar feature of this mammalian test is that the early death of treated mice is not due to a rise in metabolic rate even with more prolonged periods of treatment (up to three weeks) but to their increased sensitivity to anoxia or carbon dioxide poisoning It has yet to be shown how highly the factors responsible for early death in this assay correlate with metabolic effects and whether such a test is in fact to be preferred to the tadpole test

PARATHYROIDS

There is no international standard for extracts of the parathyroid glands Several test methods are described by Burn[1] but it is not at present possible to compare their relative accuracies The two most promising methods would appear to be that utilising the effect of parathyroid extracts in diminishing the narcotic action of magnesium sulphate to mice and that utilising the estimation of urinary calcium in the rat[55]

The potency of Parathyroid Injection U S P XIV is assayed by its effect on the serum calcium of normal dogs and is expressed in terms of U S P Parathyroid Units Each unit represents one one hundredth of the amount of injection required to raise the serum calcium by 1 milligram per 100 millilitres within 16 to 18 hours of administration

PANCREAS

Insulin (Ordinary Insulin) The international unit of insulin is the activity of 0 0455 milligram of the international standard (pure crystalline insulin 1935) preparation The new unit was defined in relation to the 1925 crude standard of which the unit was 0 125 milligram

The two standard methods for the assay of insulin the mouse convulsion method and the rabbit blood sugar method are outlined below Details for the laboratory procedure and general references are given by Burn[1] and for a modified mouse convulsion method by Young and Lewis[56]

(i) *Mouse Convulsion Method* This uses a quantal response namely the proportion of mice exhibiting convulsions after injection with insulin under standard conditions The mice are placed in a thermostatically controlled cabinet at between 29 and 37 usually at 32 in modern work Details of a standard procedure are given in the British Pharmacopœia The error of this method was investigated by Irwin[57] who found a mean slope of 3 0 in the series of assays with which he dealt and reports fully on expected errors and the actual fiducial limits of error found in the tests A slope of 3 0 is low according to present standards the mean of a series of slopes found in the laboratories of one insulin producer in the course of recent routine work was 5 3 With Irwin s data the minimal limits of error (P = 0 95) to be expected with a test using forty mice for each sample are 62 to 162 per cent with a slope of 5 3 these limits are 76 to 132 per cent The standardisation of batches of insulin is normally carried out with a total of several hundred mice for each batch neither the manufacturers nor the licensing authorities would be content with fiducial limits of error (P = 0 95) wider than about 85 to 115 per cent and they are usually expected to be within 90 to 110 per cent of the mean estimate of potency

(ii) *Rabbit Blood Sugar Method* This method depends on the reduction in the blood sugar level of rabbits which follows the injection of insulin As at present used it involves the measurement of the initial blood sugar level and the hourly blood sugar level or the blood sugar level of pooled hourly samples for five hours subsequently Full details of a standard procedure are given in the British Pharmacopœia and of the various laboratory reagents and techniques by Burn[1] The British Pharmacopœia procedure uses the cross over design elaborated by Marks and his co workers (see Marks and Pak[58]) In this test the same rabbits are used with the standard and unknown on different days so that differences in the mean sensitivity of individual rabbits are segregated in the analysis of the test and do not contribute to the estimate of error The form of test recommended is the twin cross over test full statistical examination of which is due to Smith Marks Fieller and

Broom[59] Four groups of rabbits are used and high and low doses of the standard and unknown are each administered to one of these groups on the first day of the test On the second day of the test the group which received the high dose of the standard receives the low dose of the unknown while the group which received the low dose of the standard receives the high dose of the unknown The other two groups are crossed over in a similar fashion

The error of this type of test was investigated by Dieller Irwin Marks and Shrimpton[60] and in various papers by Bliss and Marks[5] These authors agree remarkably well in that although Bliss and Marks found higher values for the slope their error mean squares are also higher and estimates of limits of error from the two series are almost identical If the standard test with twenty animals for each sample is modified in view of the cross over procedure to ten animals for each sample five animals a group in a twin cross over test and two observations per animal the expected minimal limits of error (P = 0 95) are 82 to 122 per cent

Delayed Action Insulins Globin zinc insulin and protamine zinc insulin have a more prolonged action than ordinary insulin the degree of prolongation being greater with protamine zinc insulin There are at present no standard preparations of these modified insulins which are therefore compared with ordinary insulin using the rabbit test The test of the British Pharmacopœia requires that when the average blood sugar of the rabbits receiving the standard preparation has just returned to the initial level as judged graphically, that of the rabbits receiving protamine zinc insulin should not be more than 80 per cent of the corresponding initial value This is not an exact method of comparison and the setting up of suitable standards for the delayed action insulins is in progress which will make possible the performance of tests giving precise estimates of potency and limits of error without reference to the present standard preparation It should be noted that the amount of ordinary insulin used in preparing the delayed action forms is carefully controlled so that the present tests are tests of the delaying factor not of the unitage in terms of the international standard preparation for ordinary insulin

Gonads

Œstrogens There are two international standard preparations for the assay of œstrogenic activity œstrone and œstradiol mono benzoate The international unit is in each case the activity of 0 0001 milligram of the standard preparation The œstrone standard is intended for comparison with non esterified œstrogens and the œstradiol monobenzoate standard for comparison with benzoylated preparations It has been shown however that with the usual test methods it is

valid only to compare œstrone with œstrone and œstradiol monobenzoate with œstradiol monobenzoate since the influence of the route of administration the solvent the test-object and the particular test method used is so great that even pure crystalline steroids cannot usefully be compared with one another—[61] This has led to no great difficulties in practice because the œstrogens are usually prepared as pure substances and can be characterised physically The potency of such a preparation may therefore be checked if necessary by reference to the standards or to another pure sample of the compound being examined if it is not œstrone or œstradiol benzoate

The most frequently used assay in standardisation is the Allen Doisy test for vaginal cornification in the mouse or rat[6] This test exists in various modifications the most useful of which would seem to be the injection of the substances under test in one or two daily doses in oily solution preferably in mice and the taking of vaginal smears twice a day on the third and fourth days of the test A smear with no leucocytes and with cornified epithelial cells is scored as positive all others as negative the response is therefore quantal There is wide general agreement that the value of the slope of the log dose response line is (with few exceptions) between 5 and 6 If a slope of 5 5 is found the minimal limits of error (P = 0 95) to be expected in a test with forty mice per substance are 77 to 130 per cent

Various other tests exist many founded on the increase in uterine weight of immature or spayed animals after injection of œstrogens Of these the Astwood six hour test[63] is of interest because of its speed Immature female rats twenty-one to twenty three days old are injected with the œstrogen and the uterus weighed six hours afterwards This method seems quite accurate but unfortunately the data are not presented in such a form as to allow of the estimation of the errors of the test

Progesterone The international unit of progestational activity is that contained in 1 0 milligram of international standard progesterone

There is no method of assaying progesterone biologically from which sufficient data are available for estimating errors The McPhail test[64] gives arbitrary grades to the degree of progestational proliferation of the uterus of the œstrogen primed rabbit This test could probably be made the basis of a successful quantal or even graded response assay but since it involves histological examination of the uterus it would always be rather laborious It is described by Burn[1] Another promising test is that of Astwood[65] which utilises the deciduoma reaction in the rat and could presumably also be adapted for precise work There is no general demand for such tests however because crystalline progesterone is now produced commercially and the excretion product in human urine pregnanediol is estimated chemically

Androgens The international unit of androgenic activity is that exhibited by 0 1 milligram of international standard androsterone The same situation as discussed above with reference to the œstrogens is found when dealing with androgens There is no commercially *prepared androsterone for clinical use* the compound used almost exclusively in medicine is testosterone propionate for which no bio logical standard exists or is needed and which cannot be directly compared with androsterone The practical need for standardisation of androgens is therefore very limited

The introduction of growth in the atrophic comb of the capon was the only test recognised by the 1935 Conference on the Standardisation of Sex Hormones[66] as being sufficiently specific and precise As usually performed the test requires the injection of the androgen for three to five days The response is the increase in comb size measured in various ways by *different investigators A detailed investigation of the test by* Emmens showed that when the response is the added increase in length plus height of the comb it is linearly related to dose (not log dose) and that the variance increases with the age of the birds Basing an estimate on the average standard deviation in the three day tests an assay with twenty birds per substance would have minimal expected limits of error ($P = 0\ 95$) of about 62 to 138 per cent These limits should be reduced by using a cross over technique and further reduced if a common zero slope ratio method of assay could be employed

The capon comb test although undoubtedly the most appropriate of all tests available for standardisation requires facilities often not available to the bio assayist Various tests employing the immature or castrated rat or mouse are current the responses most frequently used are the weight increases of seminal vesicles or prostate glands under androgenic stimulation Relatively rapid tests utilising seminal vesicle weight seem to be as accurate as the more prolonged tests first evolved and two recent examples are of interest Greene and Burrill[67] use immature male rats twenty to twenty two days old and inject once with androgen Forty eight hours later the seminal vesicles are dis sected and weighed fresh The log dose response relationship is linear from 5 to 50 micrograms of testosterone propionate and calculations from Greene and Burrill s data indicate that the minimal expected *limits of error* ($P = 0\ 95$) *are 64 to 157 per cent in* a forty rat test of the usual design Mathieson and Hays[68] used castrated young male rats in a similar test Their data indicate that the limits of error in a forty rat test would be 74 to 136 per cent as long as differences between groups of rats castrated at the same time are if necessary segregated in the *analysis of variance* In two practical examples using only thirty two rats per assay the limits of error ($P = 0\ 95$) were found to be 70 to 140 per cent approximately

Relative Accuracy of Test Methods

The approximately minimal numbers of animals required to give an accuracy of 80 to 125 per cent at the 95 per cent level of significance in different tests in which groups of animals are used are shown in Table XII

TABLE XII

Approximate total numbers of animals (not litter mates) needed to achieve minimal limits of error (P=0 95) of 80 to 125 per cent in various assays

Substance	*Animal*	*Method*	*No required*
Thyrotrophic hormone	Guinea pig	Thyroid weight[12]	50
Lactogenic hormone	Pigeon	Crop gland weight[5]	65
Growth hormone	(a) Plateaued rat	Body weight	110
	(b) Hypophysectomised rat	Body weight[2]	220
Adrenocorticotrophic hormone	(a) Chick	Adrenal weight	35
	(b) Hypophysectomised rat	Adrenal weight[8]	240
Chorionic gonadotrophin	(a) Rat	Ovarian weight[69]	90
	(b) Mouse	Vaginal smear[3]	45–130
Serum gonadotrophin	Rat	Ovarian weight	25
Adrenal cortical extracts	(a) Rat	Liver glycogen	350
	(b) Rat	Total fermentable sugar	30
	(c) Rat	Liver glycogen	40
Thyroxine and iodoproteins	(a) Tadpole	Eruption of front legs	95
	(b) Mouse	Survival time	80
Insulin	(a) Mouse	Convulsions	120
	(b) Rabbit	Fall in blood sugar[69]	16*
Œstrogens	Mouse or rat	Vaginal smear[2, 61]	110
Androgens	(a) Capon	Comb growth[8]	130
	(b) Rat	Seminal vesicle weight[8]	75–130

* Cross over test

These numbers are directly proportional to the inherent precision of various methods of assay as estimated from available data It should be noted that the sixteen rabbits required on the average in insulin assays are each used twice in the cross over technique and that the precision of this method is greatly enhanced by the elimination of differences between the mean sensitivity of individual rabbits in the assay The accuracy of the assays of gonadotrophins (and doubtless other substances) is also much improved if litter mates can be used and the corresponding litter differences eliminated from the estimate of error Thus the most accurate of this group of tests when litter mates are used would seem to be the assay of serum gonadotrophin by the method employing rat ovarian weight It is rarely advisable to use less

than twenty animals in an isolated assay, however, because the fiducial limits of error widen rapidly with small numbers of observations

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CHAPTER VI

ACTION AND USES

DURING recent years the action and uses of hormones have been studied extensively using the standardised extracts and pure natural and synthetic compounds now available to the clinician Since hormone preparations do not necessarily produce the same effects in patients as they do in experimental animals their clinical use is frequently empirical The treatment of glandular underactivity consists in the administration of the correct amount of a standardised extract or synthetic compound to make good the deficiency Overactivity of a gland is corrected by the surgical removal of a part of the gland or by administering preparations which have an antagonising action for example thiouracil in hyperthyroidism Treatment with hormones must be regulated for individual patients since the correct dosage necessarily depends on the degree of hormone production of the deficient gland and on the degree of activity of other interrelated glands Hormones from the anterior lobe of the pituitary stimulate many other glands and large doses of œstrogens and other hormones can depress the activity of the pituitary The complicated interaction of the different glands and the variable response *to therapy impose difficulties and limitations in the way of* satisfactory treatment with hormones

Before commencing hormone therapy an accurate diagnosis is essential and local causes of disease must be excluded Treatment with hormones can be curative only if the diseased gland is capable of functioning on discontinuation of therapy Most hormone preparations *however act by substitution only they do not have a permanent effect* and the fact that they can inhibit the hormone production of other glands must be taken into consideration After the prolonged administration of some hormone preparations their further use is followed by a marked reduction or even absence of the physiological response The doses of hormone preparations should therefore be sufficient to correct *the deficiency only since excessive doses may make any subsequent* treatment relatively ineffective Collip and Anderson[1] showed that laboratory animals became non responsive to the thyrotrophic hormone after prolonged treatment and if the blood of these non responsive animals was injected into untreated animals these also did not respond to injections of the thyrotrophic hormone owing to the presence of antihormones *Clinically it is known that the continued use of some* hormone preparations particularly those of a protein nature such as the gonadotrophins can result in a state refractory to further injections Leathem and Rakoff[2] showed that the antihormones which develop following the administration of pregnant mares serum gonadotrophin are hormone specific and with the appearance of the hormone inhibition treatment *should be suspended or a gonadotrophin from another*

source should be administered Zondek[3] considers that antihormones are unlikely to occur with the steroid hormones or insulin

THYROID

The thyroid gland intensifies the action of the intracellular metabolic enzymes thus increasing tissue metabolism It has a specific affinity for iodine and this element in addition to the thyroid hormone thyroxine must be considered in the treatment of thyroid disorders A deficiency of iodine in the diet produces an enlargement of the thyroid gland This condition is sometimes known as endemic goitre because of its frequent occurrence in districts in which there is a deficiency of iodine in the soil and water Simple goitre can be prevented by giving small doses of iodine in the form of potassium or sodium iodide which is usually added to common salt in the proportion of 1 part in 5000 to 20 000 of salt Treatment with iodine itself is not so satisfactory but some workers have obtained beneficial results with small doses of thyroid

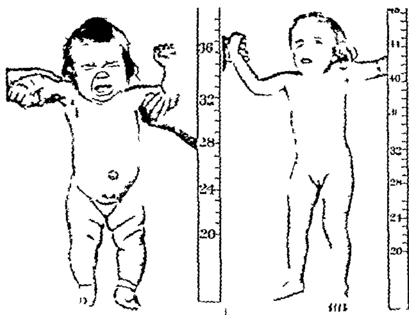


FIG 25

CRETINISM

Due to congenital deficiency of thyroxine The patient is shown at 20 months (l ft) just before the beginning of treatment by the continuous administration of thyroid and at 3 years

Hypothyroidism A deficient secretion of the thyroid hormone, or hypothyroidism may occur at any age and may be due to injury of the gland either by accident or disease Occasionally it may occur after the removal of too much of the gland in operations for hyper thyroidism In children the thyroid gland may be deficient from birth when the disease is known as cretinism if however, thyroid deficiency occurs in young children it is called infantile myxœdema

The symptoms of cretinism which may not be observed immediately are a slow gain in weight retarded mental development and a distur bance in bone formation which can be demonstrated radiologically



FIG 26
TYPICAL MYXŒDEMA
Due to underactivity of the thyroid Hair brittle and coarse skin puffy and dry general lethargy due to slowing down of the metabolic processes

Cretins are always dwarfed persons with a sallow complexion puffy eyelids and a dry skin Thyroid if given early promotes normal growth and physical development but mental development frequently remains retarded owing to the damage to the brain which has taken place during embryonic life The dose of thyroid must be adjusted to the individual needs of the patient approximately 30 milligrams (½ grain) daily may be sufficient for infants and 60 milligrams (1 grain) or more for children of four to twelve years of age Normal growth can be restored in infantile myxœdema if treatment is given early

Thyroid deficiency in adults or myxœdema was first described by

Gull in 1873 and is sometimes known as Gull's disease The name myxœdema (mucous œdema) was given to it by Ord in 1878 Persons with hypothyroidism usually have a placid drowsy expression with slow speech and intellectual processes The features are coarse and the skin thick In contrast to persons with hyperthyroidism they have a small appetite and constipation is common The treatment of myxœdema with thyroid produces excellent results and the effects of treatment appear within a week As with all other hormone deficiency states the dose required varies considerably with the patient Treatment is commenced with a dose of 60 milligrams (1 grain) daily and this is gradually increased until the symptoms disappear A dose of 0 2 gramme (3 grains) daily is sufficient for most patients but larger doses may be required It is important to increase the dose slowly because the thyroid hormone requires at least twenty four hours to take effect and cumulative effects may occur To guard against these thyroid therapy is sometimes omitted every fourth week Treatment is best controlled by estimation of the basal metabolic rate or more easily by determination of blood cholesterol[4]

Thyroxine is sometimes administered in the treatment of hypothyroidism as thyroxine sodium but this appears to have no advantages when given by mouth over thyroid preparations L Thyroxine sodium which is available in tablets containing 0 05 and 0 1 milligram equivalent respectively to about ½ and 1 grain of thyroid is more active than D thyroxine sodium and is therefore preferable to the racemic thyroxine sodium described in the British Pharmacopœia 1932 Thyroxine sodium is also administered by intravenous injection as an aqueous solution As with thyroid treatment with thyroxine sodium should be controlled by determining the basal metabolic rate or blood cholesterol

Hyperthyroidism The essence of treatment is to control the overactivity of the gland either by the surgical removal of a part of the gland or by drug therapy Iodine is frequently given before operations for removal since it lowers the basal metabolic rate and temporarily reduces the size of the goitre The mechanism of the action of iodine in hyperthyroidism is not clearly understood

Interest in drugs which can inhibit thyroid activity was stimulated by the observation that the administration of thiourea derivatives to laboratory animals was followed by typical signs of thyroid deficiency[5] and hundreds of different compounds have been prepared and tested in this respect[6] These compounds act by blocking the uptake of free iodine in the thyroid cells before the iodination of tyrosine The first of these compounds to be used clinically was thiourea but this has been replaced by the more active and less toxic derivatives thiouracil methylthiouracil and propylthiouracil

The administration of antithyroid drugs leads to the following series

of events (*a*) the production of thyroxine by the thyroid gland is decreased (*b*) the control of the pituitary gland by thyroxine is diminished (*c*) increased amounts of the thyrotrophic hormone are excreted by the thyroid gland and (*d*) the thyroid gland becomes hyperplastic As a result the thyroid gland remains enlarged although it is not producing an abnormal amount of thyroxine



FIG 27
TYPICAL THYROTOXICOSIS
(Exophthalmic Goitre Graves Disease)
Due to overactivity of the thyroid Eyes bulging general state of anxiety and restlessness

There is a close interrelationship between hormones and vitamins and an antagonism between vitamin A and tyrosine is known Simkins[7] used vitamin A in massive doses to treat two patients with hyperthyroidism and stated that he considered it a promising therapeutic agent for this condition

Certain chemotherapeutic agents can control hyperthyroidism but *they rarely cure the condition* surgery has the advantage that a cure can be quickly obtained A useful comparison of the results obtained with antithyroid drugs is given by Williams[8] The most potent preparations were propylthiouracil and *cyclo*propylthiouracil and Williams considers that they are the most desirable ones to use

Radioactive iodine has been used to apply radiations directly within the cells of the thyroid gland of patients with hyperthyroidism and there is no doubt that it is an effective way of producing a remis sion but an appraisal of its value as compared with other methods cannot be made until more patients have been treated over a long period

Other Uses of Thyroid Thyroid has been recommended for many conditions not directly associated with an underactive thyroid gland Obesity although seldom evidence of glandular disorders is not necessarily due to hypothyroidism and successful treatment with thyroid may be due to its diuretic action Thyroid has also been used in the treatment of a variety of gynæcological conditions such as amenor rhœa sterility abortion and menstrual disorders and there may be some justification for its use in these conditions because it is known that there is a close interrelationship between the gonads and the thyroid gland

Thyroid Preparations Murray* who first used thyroid in the treatment of myxœdema gave a glycerin extract by subcutaneous injection It was soon discovered however that thyroid preparations were effective by mouth and accurately standardised preparations of thyroid have been available for some time The potency of different preparations is usually estimated from the amount of iodine which they contain as thyroxine but biological assays are also employed by some manufacturers The doses of thyroid in the above sections refer to the powdered defatted standardised thyroid of the British Pharmacopœia and not to the fresh gland (see page 183)

PARATHYROIDS

The surgical removal of the parathyroid glands produces tetany This is due to a reduction in the blood calcium the symptoms of which can be relieved by the intravenous or oral administration of calcium salts They can also be relieved by the administration of an extract of the parathyroid glands which produce a hormone capable of use in replacement therapy The parathyroid hormone plays an important part in regulating the calcium level in the blood bones and urine but the mechanism of its action is still debatable

Hypoparathyroidism Extreme hypofunction of the parathyroid glands produces hyperexcitability of the nervous system with painful tonic spasms of the muscles The hands wrists and ankles are usually affected first spasms occurring later in the face trunk and sometimes the laryngeal muscles Tetany may be latent although typical symptoms are not present muscular spasms can be induced in susceptible individuals by constricting the upper arm with a tourniquet when a typical spasm in the hand is produced This is known as Trousseau s

of events (*a*) the production of thyroxine by the thyroid gland is decreased (*b*) the control of the pituitary gland by thyroxine is diminished (*c*) increased amounts of the thyrotrophic hormone are excreted by the thyroid gland and (*d*) the thyroid gland becomes hyperplastic As a result the thyroid gland remains enlarged although it is not producing an abnormal amount of thyroxine



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Calciferol in Tetany For the treatment of patients with chronic tetany calciferol (vitamin D) has been employed although its action on calcium and phosphorus metabolism is different from that of the parathyroid hormone Shelling[1] states that therapeutic amounts of calciferol invariably cause a positive balance of calcium and phos phorus and produce healing in the metaphyses of the bones if rickets be present whereas parathyroid hormone causes a negative calcium balance and a retardation in ossification Schultzer and Christensen[13] used large doses of calciferol to treat eleven patients suffering from parathyroid tetany and found that it had an effect on calcium metabolism similar to that of dihydrotachysterol The preparation used was an oily solution containing 300 000 units of calciferol equal to 7 5 milli grams in 1 millilitre Subsequent patients were given 5 milligrams of calciferol daily supplemented by intravenous injections of calcium lactate during the first few days There was a prompt rise in the serum calcium concentration but from one to five months elapsed before the maximal effect of calciferol was obtained When adjusted the patients were just as well under treatment with calciferol as with dihydrotachy sterol and showed no signs of tetany or other morbid conditions The final daily dose of calciferol was from 1 5 to 4 5 milligrams and the concentration of serum calcium in all patients after adjustment 9 to 11 5 milligrams in 100 millilitres Although the symptoms of tetany may be alleviated with either calciferol or parathyroid hormone it is only the former which increases the amount of calcium in the body

Hyperparathyroidism An excessive secretion of the parathyroid hormone results in an increased amount of calcium in the blood serum and a decrease of phosphorus Calcium is withdrawn from the bones which become cystic and deformed Von Recklinghausen[14] first des cribed the bone disorder which is also known as generalised osteitis fibrosa cystica It is an advanced stage of hyperparathyroidism characterised by gradual rarefaction and softening of the bones with development of cystic areas and deposition of calcium in soft tissues Hyperparathyroidism can be produced experimentally by continued overdosage of the parathyroid hormone but the cause of the over activity is not known A deficiency of calciferol is thought to stimulate hyperplasia of the parathyroids with resulting adenomata which may or may not produce the hormone If they do symptoms of hyperpara thyroidism result Treatment which consists of the surgical removal of the tumour usually gives relief *Keating and Cook*[15] *in an analysis of* twenty four cases showed that in only seven was there classic evidence of disease of the bones but calcification of the kidneys or renal calculi occurred in twenty two of the patients They emphasise that in hyper parathyroidism involvement of the urinary tract is a more common and important symptom than bone involvement and they suggest that every patient who has renal calculi should be suspected of having

sign and is used as a diagnostic test Other diagnostic tests are based on Chvostek s sign in which a slight momentary twitch is obtained by tapping the cheek over the facial nerve in front of the ear with a percussion hammer and Erb s sign in which there is increased irritability brought about by electrical stimuli Hypoparathyroidism is always accompanied by numbness of the extremities

One of the most reliable diagnostic tests is the decrease in the concentration of calcium ions in the blood serum The normal serum calcium level is 10 milligrams per 100 millilitres in tetany it may be 4 to 6 milligrams per 100 millilitres but the severity of symptoms does not always vary in proportion to the level of serum calcium Although the phosphorus content of the blood may remain unchanged the ratio of phosphorus to calcium is altered so that the amount of phosphorus is relatively increased

The administration of the parathyroid hormone alone is not a satisfactory treatment for most patients with parathyroid deficiency, and it is usually used as an adjuvant to other forms of therapy The parathyroid hormone is ineffective when given by mouth, and its effects by injection are cumulative The subcutaneous injection of 1 to 3 millilitres (100 to 300 units) of a preparation such as Parathyroid Injection (see page 176) usually results in raising the serum calcium to normal for a period of eight to eighteen hours immediately following the injection[10] For the treatment of tetany calcium salts and dihydrotachysterol and calciferol with or without the parathyroid hormone are also employed The intravenous injection of a 10 per cent solution of calcium gluconate causes an immediate rise in the calcium concentration in the serum but this rise is not maintained There is a slower but more prolonged effect with the parathyroid hormone but there is also a gradual loss of effect following continued treatment, probably due to the formation of antihormones in the blood

Dihydrotachysterol in Tetany Dihydrotachysterol (AT 10) was first observed by Holtz in 1934 to exert an antitetanic action It is used in the form of a 0 5 per cent solution in oil the dose being from 1 to 2 millilitres (15 to 30 minims) by mouth at the commencement of treatment with smaller doses for maintenance MacBryde[11] reported that a normal blood calcium level was maintained in seven patients over a period of three months to a year with doses of from 0 3 to 1 millilitre (5 to 15 minims) of dihydrotachysterol solution daily supplemented by 4 to 10 grammes (60 to 150 grains) daily of calcium lactate or calcium gluconate During treatment frequent estimations of serum calcium are necessary to avoid hypercalcæmia and regulation of the diet to contain a high intake of calcium and a low intake of phosphorus is considered important by some authorities Foods which should be avoided to maintain a low phosphorus intake include milk meat cheese potatoes and cocoa

readjustment of the equilibrium and there is probably an increased supply of adrenaline which has an antagonising action on insulin

Diabetes Mellitus Hypo insulinism or diabetes mellitus the classical symptoms of which are polyuria and loss of weight is a comparatively common illness and may develop at any age Many authorities stress obesity as a predisposing factor Patients with sugar in their urine are not necessarily diabetic The condition can be diagnosed and differentiated from renal glycosuria and other non diabetic conditions by means of the glucose tolerance test and an estimation of the amount of sugar in the blood (see page 22) Marble[18] points out that patients with benign conditions may be incorrectly diagnosed as having diabetes mellitus He finds pentosuria and fructosuria to be comparatively rare harmless conditions having no relation to diabetes mellitus and describes procedures for a differential diagnosis

Insulin Preparations Attempts to produce insulin compounds which are not destroyed by the digestive enzymes in the stomach have proved unsuccessful and although claims have been made for substances which are active by mouth insulin preparations for parenteral use remain the only therapeutically effective substances for use in the treatment of diabetes mellitus Three types of preparations are available Injection of insulin (Injectio Insulini B P) is a sterile solution of crystalline insulin hydrochloride containing 20 40 or 80 units per millilitre to avoid confusion it is sometimes called ordinary regular soluble or unmodified insulin Injection of protamine zinc insulin (Injectio Insulini Protaminati cum Zinco B P) introduced in 1936 is a sterile suspension of a compound of insulin and a suitable protamine with the addition of a trace of zinc chloride it contains 40 or 80 units per millilitre The zinc chloride increases the stability of the preparation and delays the action of the insulin probably by interfering with an enzymatic process concerned in the separation of insulin from the insulin protein precipitate which is formed in the tissue fluids Globin zinc insulin introduced in 1940 is a clear solution of a compound of insulin and globin a protein obtained from hæmoglobin with the addition of a trace of zinc chloride Mixtures of injection of insulin and injection of protamine zinc insulin are also employed Patients react to the different insulin preparations in a uniform manner but they vary in the amount required to produce similar responses owing to differing sensitivities to insulin

Action of Different Insulin Preparations The action of ordinary insulin is rapid in onset and a marked lowering of the blood sugar level occurs soon after injection giving immediate relief from the symptoms of hyperglycæmia Insulin is quickly absorbed from the site of injection through the lymphatics into the blood stream The

hyperparathyroidism Rogers[16] described two patients with hyper parathyroidism not recognised during life and without a history *suggesting osseous or renal disease The first patient had an adenoma of* the parathyroid gland and the second hyperplasia of the parathyroids Both patients showed metastatic calcification in the kidneys complicated by coexisting duodenal ulcers Treatment for the ulcer included a diet high in calcium and phosphorus and it produced in each instance an exacerbation of symptoms

Pancreas

Action of Insulin For many years after the experimental production of diabetes mellitus in dogs by von Mering and Minkowski in 1890 it was assumed that diabetes mellitus was purely of pancreatic origin being due to lack of the hormone later termed insulin The effect of insulin on carbohydrate metabolism is well known it stimulates the oxidative breakdown of glucose has a stimulating effect on the synthesis of liver and muscle glycogen from glucose and inhibits the breakdown of liver glycogen but how insulin acts is still little understood in spite of a vast amount of experimental work It has not yet been possible to determine accurately how much insulin is being produced by the pancreas or to study the factors which control the activity of the islets of Langerhans It is known that other glands are involved and that glycosuria occurs as the result of diseases of the anterior lobe of the pituitary and the adrenals A nervous factor is also concerned and as long ago as 1855 Claude Bernard by puncturing the floor of the fourth ventricle of the brain induced glycosuria in the rabbit showing that a centre in the hypothalamus was involved in the control of the blood sugar On the other hand it is possible to maintain carbohydrate metabolism in depancreatised dogs by pancreatic grafts separated from their original nerve and blood supply

Himsworth[17] in a series of articles deals with the mechanism of diabetes mellitus and discusses the role of the liver in the control of the blood sugar level the importance of the anterior lobe of the pituitary and the reason for the varying response of different patients to insulin He shows that the fasting blood sugar levels of both normal and diabetic persons are maintained within definite limits The maintenance of the blood sugar at this particular level is so important that attempts to change it by physical means are overcome by the body indicating that the blood sugar level existing at any particular time is that which approaches nearest to the optimum for the needs of the body This raises two questions (i) Why should the optimal blood sugar levels vary? and (ii) How are changes in optimal levels brought about? It has been suggested that if the ability of the tissues to utilise sugar is diminished by lack of insulin or impairment of insulin action compensation is made through an appropriate increase in the blood sugar level In abnormal conditions the nerve centres in the brain cause a

hyperglycæmia after a few days or it is becoming worse treatment with ordinary insulin should be started with 5 to 10 units night and morning After a few days the progress of the patient should be surveyed and the dose of insulin adjusted accordingly This process should be repeated until the patient is stabilised on the diet he is taking If this diet is inadequate it is raised to 150 grammes of carbohydrate and the necessary adjustment made in the insulin dose It is important not to alter the diet and the dose simultaneously During stabilisation it is advisable to control the process by means of blood sugar estimations but once the patient is stabilised urine tests are a satisfactory guide When changing from ordinary to protamine zinc insulin the initial dose of protamine zinc insulin should be half the total daily dose of ordinary insulin given in one injection before breakfast For severely ill patients ordinary insulin should not be omitted altogether until the protamine zinc insulin has begun to exert its full effect and the change should only be effected under close observation with frequent blood sugar and urine tests When a mixture of ordinary and protamine zinc insulin is administered it is important to draw the ordinary insulin into the syringe first

Vitamins and Blood Sugar There is some evidence that certain of the vitamins of the B group and ascorbic acid have an effect on the blood sugar level but this effect after injections of insulin appears to be variable Martin [4] believes that aneurine and riboflavine play a fundamental role but Demole and Silberschmidt[2] state that they have no effect on the blood sugar level in rabbits Dienst [6] studied the effects of aneurine and ascorbic acid he found that aneurine reduced the blood sugar resulting in less insulin being needed and considers that the diet of diabetics should be rich in both aneurine and ascorbic acid Kodicek[27] treated twenty patients with aneurine and insulin and showed that aneurine did not affect the fasting blood sugar or increase the response of diabetics to insulin Parr and Shipton[28] describe five patients in which brewers or bakers yeast increased the sensitivity to insulin improved the carbohydrate tolerance and diminished the amount of insulin required

Hypoglycæmia When the blood sugar level falls below 70 milligrams per 100 millilitres symptoms of hypoglycæmia may occur namely weakness and giddiness distress or even epileptiform convulsions and coma The prompt administration of at least 16 grammes (240 grains) of glucose or sucrose by mouth will relieve these symptoms and the dose should be repeated in half an hour if the hypoglycæmic effect of the insulin still persists When the hypoglycæmia is severe an intravenous injection of 10 millilitres (150 minims) or more of injection of dextrose (5 per cent) should be given Protamine zinc insulin is less likely than ordinary insulin to produce hypoglycæmia during the day and the hypoglycæmia is usually less severe than with ordinary insulin

disadvantage of ordinary insulin is that two, or perhaps three injections daily may be necessary to maintain a satisfactory control of the blood sugar level Nevertheless for more than ten years before the introduction of the newer types of insulin ordinary insulin permitted many thousands of diabetic to lead a comparatively normal life Ordinary insulin is the most satisfactory preparation for use in emergencies when a prompt action is essential

The action of protamine zinc insulin is more prolonged than that of ordinary insulin and its rate of onset is slower In severe cases of diabetes mellitus this delaying action may cause hypoglycæmic reactions which may occur with few warning symptoms Protamine zinc insulin is less regular than ordinary insulin in its action from day to day, *probably due to variations in absorption Because of its slow onset of* action it should not be used in the treatment of hyperglycæmic coma

Globin zinc insulin is a long acting insulin with a duration of effect and rapidity of onset intermediate between that of ordinary insulin and protamine zinc insulin A lower blood sugar level is obtained sooner after the administration of globin zinc insulin than after protamine zinc insulin The action of globin zinc insulin lasts about twenty four hours and with slight modifications of diet some patients with diabetes mellitus can be satisfactorily controlled by one injection a day Unit for unit globin zinc insulin has a quantitatively greater effect than protamine zinc insulin its action being slower during the first few hours after injection than that of corresponding doses of ordinary insulin but quicker than that of protamine zinc insulin Clinical results over a number of years have shown that globin zinc insulin although often a most useful preparation is not suitable for all patients with diabetes mellitus The faults of globin zinc insulin are shown particularly in severe diabetes mellitus[19] In large single doses it may cause late after noon hypoglycæmia which may be countered by adjustment of the diet moreover the effect often wanes so rapidly during the night that *heavy glycosuria appears before and after breakfast*

Mixtures of protamine zinc insulin and ordinary insulin have also been employed and Colwell *et al* [20] reported that in these mixtures an excess of ordinary insulin is necessary to produce appreciable alterations in the effect of protamine zinc insulin It was then shown that these mixtures contained new protamine zinc compounds with accelerated activity[19] Some authorities [1 22 23] consider that mixtures of protamine zinc insulin and ordinary insulin give a better control than globin zinc insulin and the use of two parts of protamine zinc insulin with one part of ordinary insulin seems to be most suitable

Dosage There is no hard and fast rule for dosage and each patient must be considered individually It is usual to study the effect of diet first *starting with 100 grammes of carbohydrate* daily If on such a diet there is no appreciable fall in the degree of glycosuria or

post anæsthetic toxic symptoms acidosis and pulmonary tuberculosis but its value in these conditions is questionable

Pituitary

The pituitary gland or hypophysis secretes a number of important hormones which stimulate or inhibit other endocrine glands The hormones of the anterior and posterior lobes differ widely in their physiological actions and are therefore discussed separately

Anterior Lobe

Overactivity of the acidophil cells of the anterior lobe of the pituitary before the bones are fully set results in gigantism and after



Fig 28
Cushing s Syndrome
(Pituitary Basoph l m)
The cl nical signs nclude adi pos ty of face neck and trunk abnormal l air growth in fe males and dusky complexion w th purple striæ

although severe attacks tend to occur without warning owing to the slow and gradual lowering of the blood sugar level to below the critical level Hypoglycæmia following protamine zinc insulin may occur much later than with ordinary insulin and at unusual times for example during the night before the next day s injection or before breakfast With small doses it may occur eight to twelve hours after the dose (*i e* before the afternoon or evening meal) and with large doses fourteen to twenty two hours after the dose (*i e* a morning dose causes hypoglycæmia in *the early hours of the following morning) Hypoglycæmia* following globin zinc insulin usually occurs late in the afternoon Spontaneous hypoglycæmia can result from tumours of the islets of Langerhans when the symptoms are the same as those associated with an *overdose of insulin These tumours are however* rare and *palliative* treatment consists in administering glucose

Diabetic Coma Diabetic (hyperglycæmic) coma is one of the emergencies in medicine and the outcome depends very largely on the speed with which adequate treatment is instituted The essentials of treatment are the immediate adminstration of full doses of insulin If the patient is actually unconscious it is usually advisable to give the insulin intravenously Dosage depends on the requirements of the individual patient Lawrence and Oakley[28] found that with three patients with diabetic coma 124 142 and 196 units of insulin adminis tered in divided doses every four hours over a period of twenty four hours removed ketosis controlled blood sugar and restored con sciousness

Insulin in Non diabetic Conditions Insulin has an impor tant application in the treatment of schizophrenia Its use followed the observation of Sakel[29] that it had a quietening effect in morphine addicts deprived of opiates and when unintentional severe hypoglycæmic shocks occurred there were remarkable mental changes The effects of insulin were tried in the treatment of schizophrenia and although other methods of treatment such as electro convulsive therapy and the use of leptazol which stimulate the central nervous system directly are also used insulin coma therapy is widely employed either alone or combined with convulsion therapy for this purpose The treatment consists of four phases in the first of which increasing doses of insulin are administered The second phase is characterised by shocks on each treatment day the third consists of rest periods and in the fourth small doses of insulin are given and the hypoglycæmia interrupted early[30]

It is known that some diabetic patients under treatment with insulin gain rapidly in weight, and insulin in small doses (5 to 10 units twenty minutes before meals)[31] has been used in non diabetic patients to stimulate the appetite Insulin has also been used in the treatment of

a pure form and clinical treatment with them has been disappointing The effects produced in laboratory animals are not necessarily reproduced in humans and the relative activities of samples from different species of animals vary considerably The action and uses of the following hormones or fractions will be considered growth lactogenic adrenocorticotrophic thyrotrophic and gonadotrophic

Growth Hormone The growth factor of the anterior lobe of the pituitary stimulates the anabolic phase of protein metabolism and bears a similar relationship to protein metabolism as that of insulin to carbohydrate metabolism Overproduction of the growth hormone is

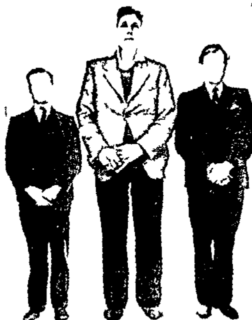


Fig 30

Gigantism

The result of oversecretion of the growth hormone before the bones are fully set The person on the right is six feet three inches

responsible for gigantism and acromegaly The use of a standardised growth hormone preparation in the treatment of dwarfed children has given poor results[33 34 3] Two millilitres (400 growth units Collip) are given daily by intramuscular injection and treatment with rest

ossification is complete in acromegaly Overactivity of the basophil cells produces a condition known as Cushing s syndrome or pituitary basophilism which in many patients is accompanied by hyperplasia or adenomata of the adrenal cortex Clinical differentiation between overactivity of the acidophil cells and of the basophil cells is difficult

Underactivity of the anterior lobe (hypopituitarism) in children is the cause of certain types of dwarfism In adults it results in Simmonds disease or pituitary cachexia Frohlich s syndrome or dystrophia adiposo genitalis in which there are abnormal deposits of fat and undeveloped sexual organs is a form of hypopituitarism

Although many physiologically active fractions have been isolated from the anterior lobe of the pituitary gland few have been obtained in

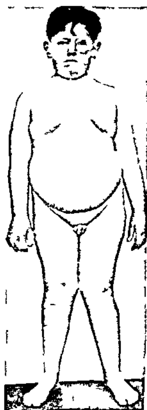


Fig 29

Fröhlich s Syndrome

Character sed by general adiposity particularly of the breasts short s ght and marked knock knees

that the administration of certain cortical hormones will cause remission of symptoms in rheumatic diseases has led to the use of the adrenocorticotrophic hormone in the experimental treatment of rheumatoid arthritis and rheumatic fever[38]

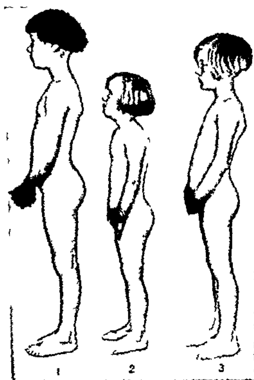


FIG 32

DWARFISM

Caused by undersecretion of the gro th hormone (1) A normal girl ag d 9 (2) the pat ent aged 9 (3) the patient s s te aged 7

Thyrotrophic Hormone The thyrotrophic hormone stimulates the production of thyroxine by the thyroid gland thus increasing the basal metabolic rate Thyroid involution, which follows prolonged thyroid administration, is probably due to inhibition of the production of the thyrotrophic hormone Bodart and Fellinger[39] showed that the concentration of the thyrotrophic hormone is increased in the blood of patients with hypothyroidism and Scowen[40] found that the hormone does not produce a rise in the basal metabolic rate in myxœdematous patients and that a refractory tate frequently occurs after about ten days treatment due to the formation of antihormones (see page 138)

intervals has to be continued for a period of up to twelve months It is of no value when the bones have fully set



FIG 31

ACROMEGALY

Oversecretion of the growth hormone after the bones are fully set causes enlargement of feet hands and jaws with coarsening of the features The patient is shown before the onset of acromegaly and a few years later

Lactogenic Hormone The lactogenic hormone prolactin is also known as the luteotrophic hormone It stimulates lactation and is secreted in increased amounts during pregnancy Desclin[36] considers that the lactogenic hormone plays an important part in sex physiology being responsible for the persistence of the corpora lutea and secretion of progesterone during pregnancy He confirms the work of Evans *et al* [37] that in addition to being a hormone of lactation it also regulates the cycle of pregnancy and he suggests that more attention should be given to it when the luteal function requires stimulation Clinically its use to increase lactation has proved disappointing

Adrenocorticotrophic Hormone The function of the adreno corticotrophic hormone (ACTH) is the stimulation of hormone production by the adrenal cortex Theoretically it should find application in the treatment of hypofunction of the pituitary adrenal system and its use in Simmonds disease has been recommended The demonstration

but there is a risk of protein reactions in sensitive patients particularly with the less pure preparations Chorionic gonadotrophin has a brief action is rapidly destroyed in the body and should be given daily to be effective It should be given during the second half of the menstrual cycle and is usually administered intramuscularly

Claesson *et al* [43] studied the action of crystalline human chorionic gonadotrophin with a potency of six to eight thousand units per milligram Their observations on the biological effects are important and do not agree with the results of earlier observers whose investigations were carried out with relatively impure products Many workers have stated that the chorionic hormone does not stimulate the follicles in the ovaries but Claesson and his co workers showed that the intravenous injection of large doses of crystalline chorionic gonadotrophin (1200 units on three successive days) gave rise to increased follicular growth in the human ovary and to forced production of œstrogenic hormones The growth of the follicles is due to a synergism between the chorionic gonadotrophin administered and the intact animal s own gonadotrophic hormones This was confirmed by the fact that after injection hypophysectomised animals showed no growing follicles even with large doses of chorionic gonadotrophin Moreover when chorionic gonadotrophin was combined with small doses of serum gonadotrophin there was an extensive development of the follicles These experiments indicate that in the treatment of amenorrhœa successful results are most likely to be obtained with the use of large intravenous doses of chorionic gonadotrophin combined with serum gonadotrophin

In the absence of genital hypoplasia Peel[44] recommends the injection of 500 to 1000 units of serum gonadotrophin every third day for three days followed by 200 to 500 units of chorionic gonadotrophin daily for three days to stimulate the normal functioning of the ovary If menstruation follows the first course of injections further courses should be given on the ninth eleventh thirteenth fifteenth seventeenth and nineteenth days of the induced cycle Bishop[4 46] although not enthusiastic about treatment with gonadotrophins states that there is some theoretical justification for the following routine up to 2000 units of serum gonadotrophin is administered intravenously for seven days combined on the fifth sixth and seventh days with 1000 units of chorionic gonadotrophin by intramuscular injection The injections of chorionic gonadotrophin are then continued alone for a further four days Rydberg and Pedersen Bjergaard[47] found that the injection of 3000 units of serum gonadotrophin daily for five days followed by intramuscular injections of chorionic gonadotrophin 1500 units every other day for three injections produced ova and corpora lutea in the majority of patients suffering from amenorrhœa Large doses should however always be given with discretion since there is a danger of overstimulation of the ovaries Doses of 500 units of serum gonadotrophin daily for ten days followed by 500 units of chorionic

Gonadotrophins Clinically the *most important hormones of* the anterior lobe of the pituitary gland are the gonadotrophins or gonad stimulating hormones which stimulate the ovaries in the female and the testes in the male to secrete their natural hormones They can be extracted from the gland only with difficulty, but two standardised preparations obtained from other sources and having a somewhat similar action to the natural hormones of the anterior lobe are described in the British Pharmacopœia They are serum gonadotrophin (Gonadotrophinum Sericum) obtained from pregnant mares serum and chorionic gonadotrophin (Gonadotrophinum Chorionicum) obtained from human pregnancy urine

Serum gonadotrophin is considered to have a predominantly follicle stimulating action on the ovary In the male it stimulates the germinal epithelium of the testes Chorionic gonadotrophin formed by the chorion or lining membrane of the placenta during pregnancy has a *predominantly luteinising action In the male it stimulates the* secretion of testosterone by the testes Serum and chorionic gonadotrophins have been in use for some time but contradictory statements in the literature make it difficult to assess their clinical value Treatment with them has not proved generally satisfactory but now that more highly refined standardised preparations are available and larger doses are employed they should have a place in sex hormone therapy when the gonads are capable of being stimulated

Before treatment with the gonadotrophins is commenced an accurate diagnosis is essential Ideally this should include tests for the gonadotrophin content of the urine or blood and endometrial biopsies *over a period of at least three menstrual cycles* Pedersen Bjergaard and Tonnesen[41] describe techniques for estimating œstrogenic androgenic and gonadotrophic substances in the urine and state that hormone secretion may be regarded as potentially pathological when the following *values occur*

(i) More than 30 Rat Units of gonadotrophic hormone for women before the menopause

(ii) Less than 20 Mouse Units and more than 400 Mouse Units of œstrogenic hormone in the intermenstrual period

(iii) More than 25 Mouse Units of œstrogenic hormone for women after the menopause

(iv) More than 25 Capon Units of androgenic *hormone*

Guterman and Schroeder[4] give details of a simplified technique for the qualitative colorimetric estimation of pregnanediol

The method and timing of administration and the dosage of gonadotrophic hormones is also important Serum gonadotrophin is long acting and a single injection is as effective as five divided doses given on consecutive days although daily injections have frequently *been recommended* Intravenous administration may be employed

a condition in which there is an excessive excretion of urine a suitable dose is 1 millilitre (15 minims) daily administered one hour before retiring Diabetes insipidus can also be treated by nasal administration of pituitary (posterior lobe) powder Choay and Choay[48] who treated more than a hundred patients confirm the efficacy of the nasal administration of pituitary (posterior lobe) powder and advise the use of frequent doses (four to six daily) of 0 1 to 0 2 gramme (1½ to 3 grains)

Although extracts of the posterior lobe of the pituitary gland produce valuable effects when injected there are few diseases which can be directly associated with the under or over activity of the gland Hypo function of the gland or lesions of the middle fossa leads to diabetes insipidus and hyperfunction associated with inadequate excretion of water by the kidneys has been named diabetes tennifluis by Simpson[49]

Metz and Lackey[50] report on sixty seven patients with peptic ulcer treated by the intranasal insufflation of 40 milligrams (⅗ grain) of pituitary (posterior lobe) powder four times daily before meals No other medication was employed and regular meals omitting only the most indigestible foods were taken three times daily This however is not a generally accepted method of treatment for peptic ulcers

Adrenals

The adrenal or suprarenal glands are associated with the name of Addison who first observed the clinical effects of destructive disease of the glands which he considered were essential to life The adrenal glands contain two distinct types of tissue which are structurally and functionally as different as the thyroid and parathyroids It is now known that the cortex is essential to life but that the medulla may be removed without any serious effects

Adrenaline The chief hormone of the medulla adrenaline produces effects which are similar to those obtained by stimulating the sympathetic nervous system When injected intravenously it causes a rapid rise in blood pressure largely due to constriction of all the blood vessels innervated by the sympathetic nervous system together with acceleration of the heart beat dilatation of the pupil inhibition of movement of the stomach intestine and bladder and the liberation of sugar from the liver Adrenaline and related substances are therefore termed sympathomimetic compounds Adrenaline disappears rapidly from the blood stream and its action lasts for only a few minutes Although small amounts of adrenaline produce profound physiological effects there is little clinical evidence that hypofunction of the adrenal medulla exists and paroxysmal hyperfunction caused by a tumour of the glands is comparatively rare Man is much more sensitive to adrenaline than laboratory animals 0 5 to 1 milligram causing a rise in systolic blood pressure whereas this amount does not affect the blood pressure in

gonadotrophin daily for ten days have been used in the treatment of sterility due to ovulatory failure

In the male serum gonadotrophin has been used in the treatment of sterility due to defective spermatogenesis the suggested dosage being 1500 units of serum gonadotrophin intramuscularly weekly or twice weekly for four to six weeks, followed by 500 units once weekly for three or four weeks For the treatment of cryptorchidism the best age for the institution of stimulation therapy is nine to ten years and provided there is no anatomical obstruction testicular descent is frequently brought about by the administration of 500 units of chorionic gonadotrophin twice weekly For *primary eunuchoidism chorionic* gonadotrophin in doses of 500 units twice weekly for a period of four to six weeks sometimes produces hypertrophy of the interstitial tissue of the testis resulting in increased secretion of testosterone with con comitant improvement in the secondary sex characteristics, including growth of the genitalia and deepening of the voice Frohlich s syndrome has been treated with chorionic gonadotrophin 500 units two or three times weekly for several weeks This is sometimes effective in the male but less so in the female

Posterior Lobe

Extracts of the posterior lobe of the pituitary when injected produce a gradual and prolonged rise in blood pressure and powerful contrac *tions of the uterus They also have an antidiuretic action and increase* intestinal tonicity and peristalsis Three preparations of the posterior lobe of the pituitary gland are described in the British Pharmacopœia, injection of pituitary (posterior lobe) (Injectio Pituitarii Posterioris) a sterile aqueous extract of the posterior lobes of mammalian pituitary bodies standardised to contain 10 units (oxytocic) in 1 millilitre injec tion of vasopressin (Injectio Vasopressini) a sterile aqueous solution containing the pressor and antidiuretic principles standardised to con tain 10 units (pressor) in 1 millilitre and injection of oxytocin (Injectio Oxytocini) a sterile aqueous solution containing the oxytocic principle standardised to contain 10 units (oxytocic)in 1 millilitre

The two fractions oxytocic and pressor, have not been highly puri fied but are used instead of the whole extract when specific actions are required Injection of pituitary (posterior lobe) and injection of vasopressin are used in obstetrics to stimulate uterine contractions and to control post partum hæmorrhage After an abdominal operation they assist the intestine to recommence peristalsis and cause evacuation of the bowels Extracts of the posterior lobe should never be given before parturition if there is a possibility of an obstacle to delivery and should be administered with caution and in small doses if there are symptoms of cardiac vascular or renal disorder The dose varies from 0 2 to 1 millilitre (3 to 15 minims) intramuscularly Generally 0 2 millilitre (*3 minims*) *is* employed before delivery In diabetes insipidus

of adrenal cortical overactivity and in distinguishing between adreno-cortical tumours and Cushing's syndrome (pituitary basophilism)[56, 57]

Adrenal cortical extracts were first thought to contain a single hormone which Hartman[58] called cortin Many crystalline fractions have however been isolated and synthetic compounds prepared which indicate that the adrenal cortex exerts many different actions The most important hormones in the cortical extracts are deoxycortone and cortisone

Adrenal cortical extracts are particularly recommended for the treatment of adrenal crises with or without deoxycortone acetate[59] During the acute state of crisis up to 60 millilitres (2 fluid ounces) or more daily by intravenous or intramuscular injection is administered with large doses of normal saline and dextrose to restore the normal blood volume As the patient's condition improves the dose is reduced slowly until the patient is finally maintained on injections of deoxycortone acetate every few days with the supplementary administration of sodium chloride daily Injection of suprarenal cortex (Injectio Suprarenalis Corticis B P C) will correct the deficiency in Addison's disease increase the serum concentration of sodium and chloride and cause a drop in the serum potassium

Deoxycortone Acetate Although injection of deoxycortone acetate does not give complete replacement therapy it is used with success in many patients with Addison's disease and it is cheaper than natural extracts In a discussion at the Royal Society of Medicine Simpson[60] confirmed the value of deoxycortone acetate in the treatment of Addison's disease and considered that 5 milligrams is equivalent to 10 millilitres of injection of suprarenal cortex Wilkinson[61] stated that deoxycortone acetate was more satisfactory than injection of suprarenal cortex and 5 milligrams was equivalent to 20 millilitres of the natural extract Soffer[59] states that the daily dose should not exceed 5 milligrams ($\frac{1}{12}$ grain) unless adequate biochemical control is available

During treatment with deoxycortone acetate an excess of sodium chloride should be avoided and adequate potassium should be administered Gordon[6] suggests that sodium salts should be given sparingly and should not exceed 5 grammes (75 grains) daily in addition to that already contained in the food Œdema and hypertension are known to be affected by the salt intake and if they develop a restriction of salt is advisable Thorn and Firor[62] state that when œdema is serious increased elimination of sodium from the body can be hastened by the administration of potassium salts

Implantation When patients have been treated for several weeks with injections of deoxycortone acetate and the approximate dosage is known the most satisfactory maintenance treatment is implantation of pellets of deoxycortone acetate under the skin The method is economical the need for repeated injections is eliminated and many favourable

animals Hypodermic injection causes little increase in blood pressure since absorption is restricted by local vasoconstriction

Injection of adrenaline (Injectio Adrenalinæ B P) is a solution of adrenaline tartrate containing adrenaline 1 in 1000 A dose of 1 millilitre (15 minims) may be used in conjunction with the parenteral or oral administration of dextrose in the treatment of hypoglycæmic attacks Adrenaline relaxes the smooth muscle of the bronchi and 0 5 to 1 millilitre (8 to 15 minims) of injection of adrenaline is usually effective in providing relief in asthma and in the treatment of anaphylactic and allergic reactions such as urticaria hay fever and serum sickness In severe asthmatic paroxysms the hypodermic needle may be left under the skin and 0 05 millilitre injected every minute (up to 10 millilitres) until the attack is controlled[51] To prolong the action of adrenaline it has been administered as a suspension of adrenaline ascorbate in a mixture of wool fat wax and oil (see page 183) The average dose is 0 3 millilitre (5 minims) and its effect lasts for six to eight hours[52] Adrenaline (1 in 100) is also administered in the form of a nasal or throat spray for prophylaxis and treatment in asthma Intracardiac injection of adrenaline directly into the cavity of the heart preferably the right auricle is used in the resuscitation of patients in whom the heart s action has apparently ceased through drowning poisoning or anæsthetic accidents It is contra indicated when chloroform has been used since the adrenaline may cause ventricular fibrillation and it is not recommended in the treatment of shock[53]

Solution of adrenaline hydrochloride (*Liquor Adrenalinæ Hydrochloridi* B P) contains adrenaline 1 in 1000 and is used locally as a hæmostatic and with local anæsthetics to localise and prolong their action and reduce hæmorrhage In dentistry the concentration used is usually adrenaline 1 in 30 000

Cortical Extracts Adrenal cortical deficiency usually attributable to tuberculosis or atrophy of the adrenal glands, produces Addison s disease characterised by pigmentation hypotension loss of weight and anorexia with a disturbed sodium, potassium and carbohydrate metabolism Hyperfunction of the adrenal cortex or hypercorticalism produced by a tumour of the adrenal cortex or by a pituitary basophil tumour which stimulates an increased secretion of the cortical hormone leads to sexual precocity feminisation in men and virilism in women The hormones of the adrenal cortex and the gonads are closely related chemically and biologically The ovarian hormone progesterone can prolong the life of adrenalectomised animals and androgens and œstrogens can be obtained from the adrenal cortex

Urinary excretion products called 17 ketosteroids can be estimated colorimetrically[54] and the relative amounts present compared with normal levels[55] are useful in the differential diagnosis of various forms

Deoxycortone Acetate in Shock There is experimental evidence that the symptoms of shock are partly due to cortical insufficiency but there is insufficient clinical evidence to justify the use of cortical preparations in the prevention or treatment of surgical shock[74, 75] although fœtal shock in twelve infants was satisfactorily treated with normal saline and deoxycortone acetate[76]

Gonads

The gonads comprising the ovaries in the female and the testes in the male secrete steroid hormones whose actions have been studied intensively in laboratory animals The gonads the anterior lobe of the pituitary and the adrenal glands are so interdependent that their actions should be considered together So called male and female sex hormones are secreted by the ovaries testes and adrenal glands and for the normal development of an individual there must be a balanced production of gonad hormones and of the pituitary gonadotrophic



Fig 33

Gynæcomastia

Enlargement of the breasts in the male may be due to excessive production of œstrogen or deficiency of androgen

results have been reported[60 61 64 65] The hormone requirement by implantation is about 60 to 75 per cent of that required by injection but the amount of hormone absorbed depends upon many factors including the surface area and density of the pellet[66] Pellets weighing 100 milligrams (1½ grains) release approximately 0 3 milligram (1/200 grain) daily and implanted pellets last from six to ten months The supplementary administration of sodium chloride 2 to 5 grammes (30 to 75 grains) daily provides some measure of control since the salt can be withdrawn if there are signs of overdosage or increased when the pellets are becoming exhausted

Techniques of implantation have not been standardised Strict asepsis is of course essential Some authorities recommend a simple incision through the skin placing the compressed tablets or pellets in pockets made on either side of the incision with a pair of fine, blunt ended scissors Others use a trocar and cannula of a diameter into which the cylindrical pellets just fit the pellets being placed deeply into the underlying muscle In other cases the pellets are placed in the fat of the abdominal wall or beneath the rectus sheath The operation can be performed in from seven to ten minutes and should be carried out in the theatre Smaller doses can be inserted in the inguinal region by infiltrating a small area with procaine hydrochloride and making a stab incision through the skin The pellet is then inserted into the stab wound

Sublingual Administration Deoxycortone acetate has been administered sublingually dissolved in propylene glycol (2 milligrams in 1 millilitre)[67] The dose required is three to five times that necessary by injection the solution should be retained for at least five minutes and administered several times a day[67 68 69 70 71]

Cutaneous Inunction Simpson[49] *reports satisfactory results in selected* patients with Addison s disease by rubbing the contents of one ampoule containing 20 milligrams of deoxycortone acetate in 100 millilitres of benzyl alcohol into the skin of the thigh abdomen or arm the process taking about ten to twenty minutes The dose required by this route is four times that required by intramuscular injection

Deoxycortone Acetate in Rheumatism In 1949 Lewin and Wassen[72] *described the use of deoxycortone acetate* combined with ascorbic acid in the treatment of rheumatism Although this and subsequent papers reported successful results other workers failed to confirm the efficacy of the treatment Copeman *et al*[73] reported that it was of no significant benefit in rheumatoid arthritis and in contrast to adrenocorticotrophic hormone and cortisone produced no more improvement than could be observed to follow the injection of procaine or even normal saline

(named after Doisy who with Allen introduced the vaginal smear technique) triphenylchloroethylene which has a very prolonged action on injection and D B E a similar compound which need be given only once a week

Action of Œstrogens Natural and artificial œstrogens have essentially the same physiological action They are responsible for the normal development of the reproductive organs and for the development of the secondary sex characteristics Œstrogens play an important part in the complicated physiology of menstruation (see page 43) During the first half of the cycle they stimulate the regeneration of the endometrium and render it sensitive to the action of the corpus luteum hormone progesterone which acts during the second half of the cycle Œstrogens also assist in maintaining the correct acidity of the vagina and mobilise the glycogen in the vaginal epithelium[80] A sudden withdrawal of œstrogen which normally occurs at the end of the cycle initiates menstruation The natural hormone œstradiol is excreted in the urine mainly in the form of œstrone and œstriol both of which possess œstrogenic activity Although œstrogens are also produced in males[81] large amounts being excreted in the urine of stallions little is known of their function in males They appear to act synergistically with androgens[82] but when used in the treatment of prostatic carcinoma they have either a direct action on the malignant cells or inhibit the action of androgens produced by the testes[83] Large doses of œstrogens inhibit the action of the anterior lobe of the pituitary and produce symptoms similar to those produced by hypophysectomy

Methods of Administration The natural and artificial œstrogens have been administered by mouth by injection as a solution in oil a suspension of fine crystals or solution in water by percutaneous application in a water miscible ointment or in a suitable organic solvent by vaginal or rectal insertion as pessaries or suppositories and by the subcutaneous implantation of tablets or pellets

The most effective way of administering natural œstrogens is by the injection of an ester such as œstradiol monobenzoate Œstrone may be administered by mouth and is useful when a small dose will suffice Percutaneous or vaginal applications are reserved for use when a high local concentration is desired and the implantation of tablets is a surgical procedure which is used only when it is desired to produce a continuous and prolonged systemic action the complete absorption of a 100 milligram (1½ grain) tablet of stilbœstrol taking about eight to nine months and a 100-milligram (1½ grain) tablet of œstradiol up to two years

Artificial œstrogens are as potent by mouth as by injection and parenteral administration is rarely if ever necessary They have been given as a compound tablet with hexobarbitone (16 milligrams) and

good results with a lower incidence of nausea are claimed with the use of hexœstrol phenobarbitone[84]

Table XIII gives the relative doses of the œstrogens The figures are approximate only and vary according to the condition to be treated and the response of the patient

TABLE XIII

RELATIVE DOSES OF ŒSTROGENS

Œstrogen	*Dose*
Œstrone	2 milligrams daily by mouth
Sodium Œstrone Sulphate	3 milligrams daily by mouth
Œstradiol Dipropionate	*5 milligrams every five days by injection*
Œstradiol Monobenzoate	5 milligrams every three days by injection
Ethinylœstradiol	*0 1 milligram daily by mouth*
Stilbœstrol	1 milligram daily by mouth
Dienœstrol	4 milligrams daily by mouth
Hexœstrol	18 milligrams daily by mouth
Dehydroxydoisynolic Acid	5 milligrams daily by mouth
Triphenylchloroethylene	100 milligrams daily by mouth
D B E	300 milligrams weekly by mouth

Toxicity The natural œstrogens are unlikely to produce any toxic effects in the doses normally employed Artificial œstrogens sometimes produce mild nausea and vomiting which usually pass off after a few days of treatment The toxicity of artificial œstrogens is related to the dosage employed and some workers believe that any side effects are closely associated with the œstrogenic potency of the preparation and cannot be dissociated from it[85] Although there is evidence that dienœstrol and hexœstrol are specifically less toxic than stilbœstrol it seems likely that the main reason why hexœstrol only rarely produces toxic effects is due to its relatively low potency There has been a *tendency in the past to give excessive doses of artificial œstrogens* and this would account for some of the untoward reactions which have been reported Artificial œstrogens are best given late at night so that the patient may sleep through the period when toxic effects are most likely to occur Nausea and vomiting are never encountered in pregnant women and very rarely in men

Clinical Applications Œstrogens are of proved value in the treatment of menopausal symptoms and inflammatory states of the vagina after the menopause to prevent breast engorgement and inhibit lactation and in the treatment of gonorrhœal vulvovaginitis in children They are useful in certain patients with delayed puberty secondary amenorrhœa and dysmenorrhœa *In the male they give relief in* prostatic carcinoma

Menopausal Disorders The vasomotor and psychic disturbances which are so frequently found at the menopause are due to declining œstrogen secretion by the ovaries and can, in most patients be controlled by the administration of either natural or artificial œstrogens The menopausal woman is very sensitive to œstrogens and the dosage used should be minimal and reduced over a period of months Bishop[45] recommends 0 25 milligram ($\frac{1}{250}$ grain) of stilbœstrol daily for a fortnight and if there is no improvement in symptoms an increase in the dose to 0 5 milligram ($\frac{1}{120}$ grain) daily for a further fortnight If necessary the dose may be further increased to 1 milligram ($\frac{1}{60}$ grain) daily during the third fortnight When an improvement is noticed the daily dose is reduced by 0 25 milligram ($\frac{1}{250}$ grain) every fortnight if there is no return of the symptoms Because of their effect on the vaginal mucous membranes œstrogens are of value in the treatment of the menopausal sequelæ senile vaginitis and kraurosis It is usually necessary to give high doses and an accurate diagnosis is essential to exclude early malignant disease Treatment may consist of tablets of stilbœstrol 1 to 5 milligrams ($\frac{1}{60}$ to $\frac{1}{12}$ grain) daily or injections of 5 milligrams ($\frac{1}{12}$ grain) of œstradiol monobenzoate weekly for one month Œstrone pessaries 0 1 milligram ($\frac{1}{600}$ grain) or an ointment containing natural or artificial œstrogens also help to clear up these conditions[44, 86]

Excessive Uterine Hæmorrhage It is possible to stop bleeding in patients with menorrhagia which has persisted for some time by giving high doses of stilbœstrol commencing with 16 to 20 milligrams ($\frac{1}{4}$ to $\frac{1}{3}$ grain) during twenty four hours, and then 5 milligrams ($\frac{1}{12}$ grain) daily for three weeks with four injections of progesterone 10 milligrams ($\frac{1}{6}$ grain) on alternate days in the next week which will be followed by bleeding which is normal in amount Repeated courses of 5 milligrams ($\frac{1}{12}$ grain) of stilbœstrol daily for three weeks followed by progesterone may tide over a difficult phase of metropathia[87] Indeed it may be possible to achieve this with the progesterone course alone repeated every twenty eight days

Prevention of Breast Engorgement Many workers have described the use of natural and artificial œstrogens to inhibit lactation Lactation depends on the level of œstrogens progesterone and gonadotrophins at parturition and also on the secretion of the lactogenic hormone by the anterior lobe of the pituitary It is maintained by the mechanical process of suckling and when established œstrogens even in high doses will not inhibit lactation They are however most useful in preventing painful engorgement of the breast in non nursing mothers[88, 89] Tablets of stilbœstrol 16 milligrams ($\frac{1}{4}$ grain) on the first day 10 milligrams ($\frac{1}{6}$ grain) on the second day and 5 milligrams ($\frac{1}{12}$ grain) daily for the rest of the first week are recommended Prescott and Basden[79] consider that parenteral treatment with hexœstrol dipropionate is more effective than hexœstrol or stilbœstrol by mouth or by injection They found

that in most patients a single injection of 12 5 milligrams (⅕ grain) given within three days of delivery was sufficient to suppress lactation

Gonorrhœal Vulvovaginitis in Children The object of treatment is temporarily to convert the vaginal mucosa from the child to the adult type, thereby making it resistant to the gonococcus Brown[90] states that there is a wide variation in the dosage and the use of œstrogens is not advised after the age of ten years Pessaries prepared with a glycerin gelatin basis have been recommended[91]

Œstrogens and Cancer Œstrogens are in some respects chemically related to the carcinogenic hydrocarbons and their prolonged administration to highly susceptible strains of mice is sometimes followed by malignant changes[9] When tested for carcinogenesis by painting on the skin however they are not carcinogenic They are carcinogenic only under special conditions usually in organs concerned with reproduction Spontaneous mammary carcinoma is known to occur in certain highly tumour sensitive strains of mice and its development is dependent on three factors (1) a genetic factor (2) a factor possibly a virus which is passed on in the milk (3) an exciting agent or œstrogen In humans there is no evidence that œstrogens used in therapeutic doses are likely to produce cancer

Use in Prostatic Carcinoma An interesting and rather unexpected use of œstrogens is in the treatment of prostatic carcinoma It had been known for some time that when the testes were removed the prostate gland decreased in size The use of œstrogens as a means of neutralising the action of testosterone or producing physiological castration was first suggested by Huggins *et al* [93, 94, 95] It was soon shown that the artificial œstrogens had the same action as natural œstrogens in this condition and most of the published work on the treatment of prostatic carcinoma deals with the use of stilbœstrol although hexœstrol and dienœstrol have also been employed Huggins[96] summarises his results after five years and a review of the chemotherapy of cancer is given by Haddow[97] Œstrogens are a valuable palliative measure they do not cure the condition but there seems little doubt that under treatment the foci of the disease may become quiescent life can be prolonged and rehabilitation can be achieved[98] Surgery is still considered the procedure of choice when the original growth can be removed completely and œstrogens are best reserved as a post operative measure following radical surgery and for the relief of symptoms in terminal cases of inoperable prostatic carcinoma Œstrogens are of no value in the treatment of simple enlargement of the prostate gland

The mode of action of the œstrogens is not clearly understood a change in the level of the serum acid phosphatase indicates that the metabolism of the malignant cells is disorganised[99] and Fergusson and

Pagel[100] have shown that œstrogen therapy does bring about a reduction in the number and size of tumour cells Œstrogens may also act through the anterior lobe of the pituitary by depressing the activity of the interstitial cells of the testes The dosage varies considerably usually a high dosage up to 16 milligrams ($\frac{1}{4}$ grain) or more daily is recommended which frequently results in the mild gynæcomastia considered desirable as an indication that treatment is proving effective

Use in Mammary Carcinoma Some success has been achieved by the empirical treatment with œstrogens of mammary carcinoma in elderly patients but it is too early to assess their value in this condition Binnie[101] believes that stilbœstrol combined with radiation therapy gives better results than if either is used alone Of 168 patients reported by various workers[10] forty one improved and in six of these the improvement was spectacular The dosage varied from 1 to 16 milligrams ($\frac{1}{60}$ to $\frac{1}{4}$ grain) of stilbœstrol daily but Beaumont and Dodds[85] state that in responding cases 1 to 5 milligrams ($\frac{1}{60}$ to $\frac{1}{12}$ grain) daily will usually suffice to bring about relief

PROGESTOGENS

Progesterone the naturally occurring progestogen is secreted by the corpus luteum in the second half of the menstrual cycle and by the placenta and chorionic villi during pregnancy It converts the proliferative endometrium to a secretory phase and assists in the maintenance of pregnancy Progesterone is excreted in the urine as an inactive water soluble compound of pregnanediol and the amount excreted is a measure of the production of progesterone by the corpus luteum and the placento chorionic system Excretion begins about twelve days before the onset of menstruation (a day or two after ovulation) and reaches a peak about five days later During pregnancy the output of progesterone and therefore pregnanediol increases very considerably Progesterone is essential for the development of the premenstrual endometrium maintenance of pregnancy desensitisation of the uterus and diminution of its power of contraction proliferation of the glandular tissue of the breast and the inhibition of ovulation during pregnancy

Progesterone is given intramuscularly as injection of progesterone (Injectio Progesteroni B P) which is a sterile solution in ethyl oleate or a suitable oil and by the implantation of tablets For oral administration ethisterone is employed usually as tablets containing 5 milligrams

Clinical Applications The main use of progesterone and ethisterone is in the treatment of abortion In habitual abortion treatment should be commenced as early in pregnancy as possible or even before the patient becomes pregnant Injection of progesterone 5 to 10 milligrams ($\frac{1}{12}$ to $\frac{1}{6}$ grain) two or three times weekly is recommended

The dose of ethisterone by mouth is approximately six times the dose of progesterone by injection the daily dosage should be divided into three or four doses and taken at intervals during the twenty four hours Six 25 milligram (⅜ grain) pellets of progesterone may be implanted as soon as a period is missed The treatment of threatened abortion is not usually so successful as the prophylactic treatment of habitual abortion and a *higher dosage is usually required thus 10 to 16 milligrams (⅙ to ¼ grain)* should be given until the cessation of bleeding followed by a gradual reduction in the dosage

Progesterone has been used in the treatment of menorrhagia when the condition is due to a deficiency of the progestational phase of menstruation as determined by uterine biopsy[86] It is also used in the treatment of metropathia hæmorrhagica in doses of 16 to 25 milligrams (¼ to ⅜ grain) daily for several days until the bleeding is under control when the dose is diminished Progesterone is not indicated in the treatment of dysmenorrhœa[45]

Androgens

The chief action of the androgens is to promote the growth of the external genital and other sexual organs they also control the secondary sex characteristics They depress the function of the anterior lobe of the pituitary and antagonise the œstrogenic hormones

The most commonly used androgen preparation is the propionic ester of testosterone administered as injection of testosterone propionate (Injectio Testosteroni Propionatis B P) by intramuscular injection An orally active derivative of testosterone methyltestosterone was introduced in 1935 and is given in the form of tablets usually containing 5 milligrams the dosage being three to four times that of testosterone propionate by injection

Steroid hormones such as methyltestosterone are also given sublingually by placing tablets under the tongue and allowing them to dissolve slowly[103] Testosterone and testosterone propionate are also *applied locally in an ointment base the strengths used being from 2 to* 25 milligrams per gramme Implantation tablets of 100 milligrams (1½ grains) are used when a prolonged sustained action is required and testosterone suppositories each containing 16 milligrams (¼ grain) of testosterone have occasionally been recommended Although synthetic compounds having an action similar to that of the natural œstrogens have been discovered no analogous synthetic androgens have so far been prepared

Clinical Applications The principal use of androgens is for the treatment of primary hypogonadism in the male for example eunuchism and eunuchoidism The dosage varies from patient to patient usually 25 milligrams (⅜ grain) three times weekly or the oral administration of methyltestosterone in doses of 30 to 60 milligrams (½ to 1

grain) daily is required to produce a maximal effect Implantation tablets of 100 milligrams (1½ grains) are employed when a very prolonged action is desired

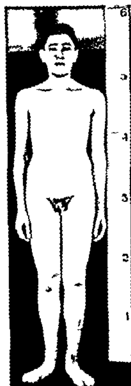


Fig 34

Eunuchoidism

Characteristics of this condition are the excessive length of limb poor muscular development high pitched voice and lack of facial and pubic hair

Methyltestosterone in doses of 20 to 50 milligrams (⅓ to ¾ grain) daily has given good results in the treatment of eunuchism and eunuchoidism[104, 105] Androgens have also been used in the treatment of selected cases of undescended testes and they are stated to be effective in relieving the subjective symptoms of the male climacteric[106] Androgens are of no value in the treatment of sterility associated with aspermatogenesis and although they have been reported to give symptomatic relief in benign prostatic hypertrophy they are not a

substitute for surgery They have given satisfactory results in a number of gynæcological complaints notably functional hæmorrhage and painful chronic mastitis and are useful in doses of 25 milligrams ($\frac{3}{8}$ grain) or more twice daily for one to three days for the suppression of lactation[107] *Methyltestosterone in doses of 25 milligrams ($\frac{3}{8}$ grain) every* four to six hours may be used twenty four hours after delivery instead of testosterone propionate to inhibit lactation Androgen therapy in women may give rise to undesirable masculinising effects and is not recommended when œstrogens may be effective

Use in Cancer of the Breast Androgens in the form of testosterone propionate by injection testosterone by implantation and methyltestosterone by mouth have been used in the treatment of carcinoma of the breast in young and middle aged women A dose of 100 milligrams ($1\frac{1}{2}$ grains) of testosterone propionate by intramuscular injection three times a week for ten weeks followed by a maintenance dose of 40 to 60 milligrams ($\frac{5}{8}$ to 1 grain) of methyltestosterone by mouth for eight weeks produced palliative results and in two patients with metastases to bone there was relief of pain a feeling of well being and general symptomatic improvement[108] Massive doses of up to 0 2 gramme (3 grains) of testosterone propionate daily produced no satisfactory improvement in six patients treated by Herrmann and Adair[109] and in two patients treated by Wyatt[110] It has been shown by several workers that androgens may be of value in the treatment of osseous metastases secondary to carcinoma of the breast[111] They have an influence on the growth processes of mammary carcinoma although clinical results have usually been disappointing but the fact that chemotherapeutic agents do have some action on the cancer cell may lead to a new approach to this problem

ALLERGY TO HORMONES

Zondek *et al*[112] found that patients suffering from typical allergic symptoms were sometimes hypersensitive to their own hormones the condition of endocrine allergy being detected by the intracutaneous injection of 0 1 milligram ($\frac{1}{640}$ grain) of steroid hormone dissolved in 0 1 millilitre ($1\frac{1}{2}$ minims) of olive oil *Tests with different hormones for* the presence of hormone allergy in six patients with keratitis rosacea showed *hypersensitivity to testosterone only Desensitisation treatment* with small gradually increasing doses 0 01 to 1 milligram ($\frac{1}{6400}$ to $\frac{1}{60}$ grain) in a series of twenty injections of hormone produced an *improvement in the condition of every patient Relief is obtained only* when the testosterone is given in low gradually increasing doses the administration of large doses sometimes causes a severe exacerbation When desensitisation has been achieved Zondek recommends the implantation of pellets containing 10 milligrams ($\frac{1}{6}$ grain) at intervals of three to six months to prevent recurrence of the allergic condition

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Injectio Insulini B P (Injection of Insulin Insulinum Insulin) is also called ordinary regular soluble or unmodified insulin It consists of a sterile solution of the specific antidiabetic principle of mammalian pancreas in water of *p*H 3 to 4 containing an antiseptic to inhibit the development of any organism introduced during use

The active principle may be prepared from finely divided fresh or frozen pancreas by extraction with acidified aqueous alcohol followed by filtration The filtrate which contains the insulin is freed from unwanted proteins by concentrating to low bulk and adding more alcohol Much of the unwanted protein material is precipitated when the concentration of ethyl alcohol is between 60 and 70 per cent and filtered off The concentration of alcohol is then increased to 95 per cent and the precipitated material collected and dissolved in water From this solution the active material is obtained by adjusting the reaction to the iso-electric point which is between *p*H 5 and *p*H 6 and filtering Instead of separating the active material from aqueous solution by the iso-electric point method it may be obtained by adding trinitrophenol and precipitating as a complex The complex is decomposed by dissolving in acidified aqueous alcohol and pouring the solution into an excess of acetone The precipitate obtained by either method is dried and powdered and the powder is dissolved in water of *p*H 3 to 4 to which the required antiseptic is added It is sterilised by filtration assayed biologically and adjusted to contain 20 40 or 80 units in each millilitre It is packed in sterile glass multiple dose containers which are sealed with rubber caps or by other methods to allow the withdrawal of doses on different occasions The labels on the containers should state the strength the date of manufacture and the date after which the injection should not be used Injection of insulin is a clear colourless liquid and should not be used if it develops a turbidity It should be stored at a temperature just above its freezing point and not exposed to temperatures above 20 Under these conditions it should retain its potency for at least two years if the reaction of the solution remains between *p*H 3 and 4 Injection of insulin is usually given by subcutaneous injection but may be given intravenously in certain circumstances The dose is determined by the physician in accordance with the needs of the patient Unless otherwise stated injection of insulin containing 20 units in each millilitre should be dispensed

Injectio Insulini Protaminati cum Zinco B P (Injection of Protamine Zinc Insulin Protamine Zinc Insulin) is a sterile suspension of a combination of insulin and a suitable protamine such as that obtained from the milt of the salmon trout together with a small proportion of zinc chloride The suspension also contains glycerin sufficient sodium phosphate to maintain the reaction between *p*H 6 9 and 7 3 and an antiseptic to inhibit the development of any organism introduced during

CHAPTER VII

PHARMACY

PREPARATIONS of hormones may be divided for pharmaceutical pur poses into two groups The first group comprises those in which the active principle is of unknown chemical constitution and includes the proteins of the parathyroid pancreas and pituitary glands and the gonadotrophins The second group comprises those in which the active principle is of known chemical constitution and includes thyroxine adrenaline and the steroid hormones of the adrenal cortex and gonads together with their artificial analogues With the first group of prepara tions the chief concern of the pharmacist is with the preservation of the potency and storage since they are usually supplied by the manufac turers ready for use With the second group there is ample scope for the application of pharmaceutical skill in presenting the active principles in the most suitable form for the particular purpose required and in ensuring maximum activity and stability

PREPARATIONS OF HORMONES OF UNKNOWN CONSTITUTION

Parathyroids The parathyroid hormone is ineffective by mouth and is usually administered intramuscularly as parathyroid injection

Parathyroid injection is official in the U S P XIV and consists of a sterile aqueous solution prepared from the fresh parathyroid glands of animals used for food by man The glands are removed immediately the animals are killed and extracted at once or kept frozen until extracted They are freed from fat and connective tissue ground and extracted The extract is sterilised preferably by filtration and ad justed to a potency of not less than 100 U S P Parathyroid Units per millilitre The injection should be packed in hermetically sealed con tainers preferably containing a single dose, and stored at a temperature not greater than 15 It may become slightly turbid or form a slight precipitate An expiry date after which it is to be regarded as unsuit able for use is not given but most manufacturers allow eighteen months The average dose is stated as 25 U S P Parathyroid Units by intra muscular injection

A parathyroid extract prepared in a somewhat similar manner was described in the British Pharmaceutical Codex 1934 but has been omitted from the British Pharmaceutical Codex 1949

Pancreas The hormone of the pancreas insulin resembles the parathyroid hormone in that it is destroyed in the alimentary tract and cannot be given by mouth In the treatment of diabetes mellitus it is administered by injection in one of the forms described below It is also used in the treatment of certain conditions by local application

Injectio Insulini B P (Injection of Insulin Insulinum Insulin) is also called ordinary regular soluble or unmodified insulin It consists of a sterile solution of the specific antidiabetic principle of mammalian pancreas in water of pH 3 to 4 containin an antiseptic to inhibit the development of any organism introduced during use

The active principle may be prepared from finely divided fresh or frozen pancreas by extraction with acidified aqueous alcohol followed by filtration The filtrate which contains the insulin is freed from unwanted proteins by concentrating to low bulk and adding more alcohol Much of the unwanted protein material is precipitated when the concentration of ethyl alcohol is between 60 and 70 per cent and filtered off The concentration of alcohol is then increased to 95 per cent and the precipitated material collected and dissolved in water From this solution the active material is obtained by adjusting the reaction to the iso electric point, which is between pH 5 and pH 6 and filtering Instead of separating the active material from aqueous solution by the iso-electric point method it may be obtained by adding trinitrophenol and precipitating as a complex The complex is decomposed by dissolving in acidified aqueous alcohol and pouring the solution into an excess of acetone The precipitate obtained by either method is dried and powdered and the powder is dissolved in water of pH 3 to 4 to which the required antiseptic is added It is sterilised by filtration assayed biologically and adjusted to contain 20 40 or 80 units in each millilitre It is packed in sterile glass multiple dose containers which are sealed with rubber caps or by other methods to allow the withdrawal of doses on different occasions The labels on the containers should state the strength the date of manufacture and the date after which the injection should not be used Injection of insulin is a clear colourless liquid and should not be used if it develops a turbidity It should be stored at a temperature just above its freezing point and not exposed to temperatures above 20 Under these conditions it should retain its potency for at least two years if the reaction of the solution remains between pH 3 and 4 Injection of insulin is usually given by subcutaneous injection but may be given intravenously in certain circumstances The dose is determined by the physician in accordance with the needs of the patient Unless otherwise stated injection of insulin containing 20 units in each millilitre should be dispensed

Injectio Insulini Protaminati cum Zinco B P (Injection of Protamine Zinc Insulin Protamine Zinc Insulin) is a sterile suspension of a combination of insulin and a suitable protamine such as that obtained from the milt of the salmon trout together with a small proportion of zinc chloride The suspension also contains glycerin sufficient sodium phosphate to maintain the reaction between pH 6 9 and 7 3 and an antiseptic to inhibit the development of any organism introduced during

use It is an almost colourless turbid liquid containing 40 or 80 units in each millilitre and should be packed labelled and stored in the manner described for ordinary insulin except that the *label should also direct* that the container be well shaken before use Injection of protamine zinc insulin is always given by subcutaneous injection The dose is *determined by the physician* in accordance with the need of the patient Unless otherwise stated injection of protamine zinc insulin containing 40 units in each millilitre should be dispensed

Injectio Insulini Globini cum Zinco (Injection of Globin Zinc Insulin Globin Zinc Insulin) is a sterile aqueous solution of a combination of insulin and the protein globin obtained from hæmoglobin together with a small proportion of zinc chloride It also contains glycerin and an antiseptic to inhibit the development of any organism introduced during use and the reaction is adjusted to about pH 3 5 It is sterilised by filtration Injection of globin zinc insulin is an almost clear, colour less liquid It has *approximately the same stability* as ordinary insulin and the same conditions of packing labelling and storage should be applied

Preservation of Insulin There has been considerable controversy about the desirability of adding antiseptics to aqueous preparations of insulin Leyton and Poulton[1] and Bennett[2] doubt the necessity of any addition and Sandor[3] states that the compulsory addition is injurious On the other hand Hartley[4] showed that whereas preparations of pH 3 to 4 are germicidal a slight increase in alkalinity nullifies the germicidal action and if no antiseptic is present converts the preparation into a culture medium

Identification of Insulin Packs To facilitate recognition of the various types of insulin the colour scheme for labels and packages described in Table XIV has been adopted by the manufacturers in Great Britain

TABLE XIV

IDENTIFICATION COLOURS FOR INSULIN PACKS

Type and Strength	*Colour of Label and Package*
Injection of Insulin	
20 units per millilitre	Buff
40 units per millilitre	Blue
80 units per millilitre	Green
Injection of Globin Zinc Insulin	
40 units per millilitre	Blue and brown
80 units per millilitre	Green and brown
Injection of Protamine Zinc Insulin	
40 units per millilitre	Blue and pink
80 units per millilitre	Green and pink

Insulin Tablets Insulin may be made into tablets using an inert substance such as lactose or dextrose as diluent The tablets should be issued in sealed sterile containers labelled with the number of units in each tablet and should yield a clear sterile solution when dissolved in water for injection

Insulin for Local Application Insulin has been used locally in the treatment of varicose ulcers either as a wet dressing or as an ointment in wool fat or ointment of wool alcohols

Anterior Lobe of the Pituitary Preparations of those hormones of the anterior lobe which are available commercially consist mainly of mixtures of the hormones in which one particular effect predominates

Growth Hormone This is available in concentrated forms usually containing traces of the thyrotrophic and gonadotrophic hormones The preparations are standardised in rat growth units and are usually supplied in multiple dose containers which should be stored in a refrigerator They should be used within six months of manufacture

Gonadotrophic Hormones Several preparations are available containing what is called the gonadotrophic hormone either alone or in various combinations and standardised in a variety of units There is little information concerning the stability of the gonadotrophic hormones and they should be kept in a cool place

Lactogenic Hormone (Prolactin Luteotrophic Hormone) This hormone is stable under ordinary conditions and may be stored at room temperature Lyons[5] reports that a 1 per cent solution at *p*H 7 6 may be heated at 100 for twenty minutes without loss of potency

Thyrotrophic Hormone Preparations of this hormone either alone or with gonadotrophic hormones are issued in multiple dose containers

Adrenocorticotrophic Hormone (ACTH) is supplied as a solution or as a sterile powder to be dissolved before use in an aqueous solvent containing an antiseptic It is stable to heat and may be stored at ordinary temperatures A method of preparation is described by Reiss and Halkerston[6] The best source is pig pituitary 1 kilogram yielding enough for 600 to 800 25 milligram ampoules A crude preparation is first obtained by extracting the minced fresh or frozen glands three or four times with acetone and hydrochloric acid diluting the solution thus obtained with acetone and filtering A further yield is obtained from the filtrate by treatment with trinitrophenol followed by ammonia and a large excess of acetone The combined precipitates constitute the crude preparation which is free from the gonadotrophic growth and thyrotrophic hormones but contaminated with a small amount of the

lactogenic hormone and with the hormones of the posterior lobe The lactogenic hormone is removed by precipitation with sodium chloride at pH 3 The posterior lobe hormones are removed by extraction with ammoniacal methyl alcohol in which they are more soluble than adrenocorticotrophic hormone The described method gives three apparently distinct fractions of the adrenocorticotrophic hormone all nearly completely free from oxytocic activity The fractions are washed with acetone dried assayed and packed into ampoules The powders may be heated at 180 to 200 *in vacuo* without loss of activity but with loss of solubility this effect may be useful in reducing the very rapid rate of excretion of the hormone Concentrated solutions may be filtered through a Seitz filter or irradiated by a 30 watt ultraviolet lamp without loss of activity

Pituitary like Gonadotrophins Two gonadotrophic hormones from other sources have been described as pituitary like because of their close resemblance in action to the follicle stimulating and luteinising hormones They are chorionic gonadotrophin from human pregnancy urine *and serum gonadotrophin from the serum of pregnant* mares

Gonadotrophinum Chorionicum B P (Chorionic Gonadotrophin) is obtained from the urine of pregnant women The urine is freed from inert material alcohol is added and the reaction is adjusted to pH 5 The precipitated material is collected washed and dried It is assayed biologically and diluted if necessary with a neutral powder to a potency equal to that of the standard preparation It is a white or fawn coloured powder soluble in water and provided it is stored in hermetically sealed containers at a temperature not higher than 20 retains its potency for twelve months Containers must be labelled with the total number of units and the date of manufacture Injection of chorionic gonadotrophin (Injectio Gonadotrophini Chorionici B P) is prepared immediately before use by dissolving the contents of a sealed container in the required amount of water for injection containing 0 5 per cent of phenol The dose of chorionic gonadotrophin is 100 to 500 units by intramuscular injection

Gonadotrophinum Sericum B P (Serum Gonadotrophin) is prepared from the blood taken from pregnant mares between the sixtieth and seventy fifth days of pregnancy in a manner similar to that used in preparing chorionic gonadotrophin It is assayed biologically and adjusted to a potency equal to that of the standard preparation It is a white powder, soluble in water and is subject to the same storage and labelling requirements as chorionic gonadotrophin Injection of serum gonadotrophin (Injectio Gonadotrophini Serici B P) is prepared immediately before use by dissolving the contents of a sealed container in the

required amount of water for injection containing 0 5 per cent of phenol The dose of serum gonadotrophin is 200 to 1000 units by intramuscular injection

Mixtures of Gonadotrophic Hormones A mixture of chorionic gonadotrophin and an extract of the anterior lobe containing the follicle stimulating hormone is available as a dry powder and in liquid form It should be stored in a cool place The powder retains its activity for several years but the liquid preparation should be used within six months of the date of manufacture

Posterior Lobe of the Pituitary Extracts of the posterior lobe contain the oxytocic pressor and antidiuretic principles The oxytocic principle can be almost completely separated from the pressor and antidiuretic principles and all three preparations (total extract oxytocic extract and pressor and antidiuretic extract) are used as pharmacological or therapeutic tools rather than for replacement therapy

Injectio Pituitarii Posterioris B P (Injection of Pituitary (Posterior Lobe) Extractum Pituitarii Liquidum Pituitary (Posterior Lobe) Extract Pituitary Extract Posterior Pituitary Injection) is the complete aqueous extract of the posterior lobes of mammalian pituitary glands The glands are removed from the animals as soon as possible after death and immediately frozen The posterior lobes are separated subdivided and either used at once or dehydrated and powdered The extract is prepared by immersing the frozen material or the dry powder in distilled water acidified to *pH 3 to 4* with acetic acid and sufficiently hot to coagulate proteins and destroy autolytic enzymes After filtration the extract is assayed and if necessary diluted to a potency of 10 units (oxytocic) per millilitre and the reaction again adjusted to *p*H 3 to 4 The extract is sterilised either by filtration before being placed in glass containers or by heating in an autoclave after sealing in *the containers The containers should be sealed glass ampoules or* multiple dose containers When the injection is sterilised by filtration or when it is supplied in multiple dose containers it must contain a bacteriostatic Injection of pituitary (posterior lobe) is a clear colourless liquid with a faint odour Provided its reaction remains between *p*H 3 and 4 and it is stored just above its freezing point it should retain its activity for eighteen months It should be labelled with the number of units (oxytocic) in a millilitre the date of manufacture and the date after which it is not to be used and may also bear the number of units (antidiuretic) and units (pressor) in each millilitre The dose of injection of pituitary (posterior lobe) is 0 2 to 0 5 millilitre (3 to 8 minims) by subcutaneous or intramuscular injection

Injectio Oxytocini B P (Injection of Oxytocin Oxytocin) is a sterile aqueous solution of the oxytocic fraction of an extract of the posterior

lobe of mammalian pituitary glands adjusted to a potency of 10 units (oxytocic) in a millilitre. Any pressor activity must not be greater than that corresponding to 0.5 unit (pressor) in a millilitre. The methods of sterilising, packing, labelling and storing are similar to those for injection of pituitary (posterior lobe). The dose of injection of oxytocin is 0.5 to 1 millilitre (8 to 15 minims) equivalent to 5 to 10 units (oxytocic) by subcutaneous or intramuscular injection.

Injectio Vasopressini B.P. (Injection of Vasopressin. Vasopressin) is a sterile solution containing the pressor and antidiuretic fraction of an extract of the posterior lobe of mammalian pituitary glands adjusted to a potency of 10 units (pressor) in a millilitre. Any oxytocic activity present should not be greater than that corresponding to 1 unit (oxytocic) per millilitre. The methods of sterilising, packing, labelling and storing are similar to those for injection of pituitary (posterior lobe). The dose of injection of vasopressin is 0.5 to 1.5 millilitres (8 to 25 minims), equivalent to 5 to 15 units (pressor) by subcutaneous or intramuscular injection.

For a prolonged antidiuretic action the most suitable preparation consists of an oily suspension of the tannate of the pressor principle. A solution of the pressor principle is treated with tannic acid and the resulting precipitate suspended in arachis oil. The suspension is usually adjusted to a potency of 5 units (pressor) per millilitre and is stable at room temperature[7].

Pituitary (Posterior Lobe) Emulsion has also been used to obtain a prolonged antidiuretic action but it has several disadvantages when compared with the tannate suspension, chiefly the formation of paraffinomata, and is now seldom used[7,8]. It may be prepared according to the following formula:

Injection of Pituitary (Posterior Lobe)	40 ml
Lactic Acid	0.4 ml
Wool Fat	0.5 g
White Beeswax	0.2 g
Chloroxylenol	0.01 g
Olive Oil	a sufficient quantity

Mix the injection of pituitary (posterior lobe) and the lactic acid and concentrate *in vacuo* over sulphuric acid to 4 millilitres. Mix the remaining ingredients, using sufficient olive oil to produce 6 millilitres, in a wide mouthed, stoppered bottle and heat at 150 for one hour. Allow the mixture to cool to 40 and gradually add the pituitary concentrate, shaking between each addition to emulsify. On cooling, the emulsion solidifies and must be warmed to about 40 before administration. To avoid destruction of the active principles, care must be taken not to overheat the preparation.

Pituitary (Posterior Lobe) Powder is used as a snuff. It is prepared from mammalian pituitary bodies by immersion in several changes of acetone, drying, powdering and assaying. The powder is usually diluted with lactose. It occurs as a yellowish or greyish powder with a characteristic odour. A water miscible nasal jelly has also been used as a means of administering the antidiuretic principle of the posterior lobe

A suitable preparation may be made by dissolving 0 5 to 1 per cent of sterculia or about 3 per cent of tragacanth in a solution of vasopressin containing 10 units per millilitre and adding a preservative such as 0 5 per cent of chlorbutol It should be supplied in collapsible metal tubes fitted with nozzles suitable for nasal use

Preparations of Hormones of Known Constitution

Thyroid Although thyroxine the hormone of the thyroid gland is available as a pure crystalline substance it is usually administered as the dried gland

Thyroideum B P (Thyroid Thyroideum Siccum Dry Thyroid Thyroid Extract Thyroid Gland) is prepared from the thyroid glands of oxen sheep and pigs by drying powdering defatting and again drying It is assayed and diluted with lactose to contain 0 09 to 0 11 per cent of iodine in combination as thyroxine It occurs as a buff coloured powder and should be stored in well closed containers in a cool place It is almost invariably administered as tablets (Tabellæ Thyroidei B P) if the strength of the tablet is not prescribed 1 grain tablets should be dispensed The dose of thyroid is 30 to 120 milligrams (½ to 2 grains)

A glycerin extract of thyroid was described in the British Pharmacopœia 1898 under the title Liquor Thyroidei and had a considerable use although it has now fallen out of favour It is occasionally used to make a thyroid ointment by incorporation with ointment of wool alcohols using 1 millilitre to each gramme of ointment of wool alcohols

Thyroxine is usually administered as the sodium salt which was included in the British Pharmacopœia 1932 under the name thyroxine sodium (Thyroxinsodium) but was omitted from the British Pharmacopœia 1948 The substance described consisted of the sodium salt of racemic thyroxine which was much less active weight for weight than the naturally occurring L thyroxine L Thyroxine is now available commercially as the sodium salt and is used as tablets containing 0 05 and 0 1 milligram It is also given by injection as a solution of the sodium salt in water for injection The British Pharmaceutical Codex 1934 directed that solutions for injection should be prepared immediately before use by dissolving in water for injection New and Non official Remedies 1947 states that a solution of freshly prepared thyroxine sodium may be sterilised by immersion in boiling water Thyroxine ointment is prepared by dissolving 2 milligrams of thyroxine sodium in 1 millilitre of water and incorporating the solution in 1 gramme of ointment of wool alcohols

Antithyroid Substances used in the treatment of hyperthyroidism include thiouracil methylthiouracil and propylthiouracil They are usually administered as tablets

Adrenals The hormone adrenaline secreted by the medulla is used as a pharmacological and therapeutic tool whereas the steroid hormones of the cortex are used for replacement therapy Since the cortical hormones are similar to the natural sex hormones they are treated together under the section entitled Steroid Hormones

Adrenalina B P (Adrenaline Adrenalinum Epinephrine) is prepared from an acid extract of adrenal glands or by synthesis It is a colourless or pale buff crystalline powder sparingly soluble in water Adrenaline is stable if stored in dark glass containers neutral or alkaline solutions rapidly become red on exposure to the air For local application it is used as solution of adrenaline hydrochloride and for parenteral administration as injection of adrenaline

Liquor Adrenalinæ Hydrochloridi B P (Solution of Adrenaline Hydrochloride Epinephrine Solution) is an aqueous solution of adrenaline hydrochloride containing chlorbutol sodium chloride sodium metabisulphite and adrenaline 1 in 1000 It should be stored in small well filled containers in a cool place and protected from light It is not a sterile preparation and is intended for topical use either alone or added to other preparations

Injectio Adrenalinæ B P (Injection of Adrenaline Injection of Adrenaline Tartrate) is a sterile aqueous solution of adrenaline tartrate containing sodium chloride sodium metabisulphite and adrenaline 1 in 1000 The tartrate is more suitable than the hydrochloride for the preparation of an injection because provided the solution contains 0 1 per cent of sodium metabisulphite and the reaction is maintained at *p*H 3 6 it can be sterilised by heating in an autoclave[9] In preparing the injection the pharmacopœial method should be strictly followed since if the tartaric acid solution is too dilute the adrenaline dissolves with difficulty and if the adrenaline is added before the sodium metabisulphite it may be oxidised If rubber caps are used to close the containers they should be previously immersed in a 0 1 per cent solution of sodium metabisulphite for forty eight hours Injection of adrenaline should be stored protected from light

Other Adrenaline Preparations Numerous attempts have been made to devise preparations of adrenaline which can exert their effect over a prolonged period A suspension of adrenaline in oil prepared by triturating adrenaline with sterile arachis oil has been used usually about 0 25 per cent of adrenaline is incorporated Other preparations used for the same purpose contain combinations of adrenaline with mucic acid and with ascorbic acid

Injection of adrenaline mucate may be prepared from the following formula using the method of the British Pharmacopœia for injection of adrenaline

Adrenaline	0.1 g
Mucic Acid	0.114 g
Sodium Metabisulphite	0.1 g
Sodium Chloride	0.8 g
Chlorocresol	0.1 g
Water for Injection	to 100 ml

A preparation containing adrenaline ascorbate was described by Kennedy[10] who gave the following formula

Adrenaline	0.01 g
Ascorbic Acid	0.015 g
White Beeswax	0.02 g
Wool Fat	0.04 g
Arachis Oil	1 ml

Heat together the white beeswax, the wool fat and a portion of the arachis oil at 150° for one hour and cool. Mix the adrenaline and the ascorbic acid and powder very finely in a sterile mortar. Triturate the powders with the remainder of the arachis oil previously heated at 150° for one hour and cooled and add the mixture of waxes.

The final injection is semi-solid at ordinary temperatures and should be warmed to about 40° before use; the syringe should also be warmed to the same temperature.

Preparations for the eye include a compound lotion (Collyrium Adrenalinæ Compositum B.P.C.) and an eye ointment, a suitable formula for which is

Adrenaline	0.025 g
Chlorbutol	2.0 g
Boric Acid	0.075 g
Distilled Water	2.0 ml
Wool Fat	5.0 g
Liquid Paraffin	20.0 g
White Soft Paraffin	to 1000.0 g

Dissolve the adrenaline and the boric acid in the water and the chlorbutol in the liquid paraffin. Mix the wool fat with the white soft paraffin and incorporate the two solutions.

Adrenaline is administered as a nasal spray, either alone (Nebula Adrenalinæ B.P.C.) or combined with atropine and papaverine (Nebula Adrenalinæ et Atropinæ Composita B.P.C.). It is also applied to the nose as a compound ointment prepared according to the following formula

Solution of Adrenaline Hydrochloride	4 ml
Chlorbutol	4 g
Benzocaine	4 g
Wool Fat	12 g
Yellow Soft Paraffin	to 100 g

Mix the wool fat and yellow soft paraffin and incorporate a mixture of the chlorbutol and benzocaine in fine powder and then the solution of adrenaline hydrochloride.

For rectal application adrenaline is used as suppositories (Suppositorium Adrenalinæ B.P.C. and Suppositorium Adrenalinæ et Cocainæ B.P.C.)

Steroid Hormones In this section the method of presenting the steroid hormones from the adrenal cortex and the gonads and the artificial œstrogens are briefly reviewed In general these substances are all stable crystalline and well defined and present few problems in pharmacy Their chief interest lies in the wide range of forms in which they are used in contrast to most of the hormones previously discussed On account of this it is convenient to arrange this section according to the route of administration

Oral Administration In general the natural œstrogenic hormones lose much activity when given by mouth, and except for œstrone are seldom given by this route The artificial hormones on the other hand, lose little activity and have proved effective by mouth They are usually administered as tablets and the following are described in the British Pharmacopœia

Tabellæ Æthisteroni
Tabellæ Dienœstrolis
Tabellæ Hexœstrolis
Tabellæ Methyltestosteroni
Tabellæ Stilbœstrolis

Tablets of œstrone (Tabellæ Œstroni) are also described in the British Pharmacopœia

Sublingual Administration Those substances which are readily absorbed by the rich network of capillaries under the tongue can be introduced directly into the systemic circulation without risk of destruction by the enzymes of the alimentary tract or the liver Among the substances thus administered are ethisterone and methyltestosterone, which are supplied in special tablets for sublingual administration They should be allowed to dissolve slowly under the tongue as little as possible of the saliva being swallowed Deoxycortone acetate has also been administered sublingually as a 0 2 per cent solution in propylene glycol

Percutaneous Administration The sex hormones are readily absorbed through the intact skin when applied as ointments or solutions Several ointment bases have been tried and the most suitable appear to be hydrous ointment and ointment of wool alcohols although an oil in water base such as emulsifying ointment is also used Stilbœstrol and dienœstrol are also absorbed through the skin particularly through the breasts and are frequently administered in this way

A selection of suitable formulæ are given below

Dienœstrol Cream

Dienœstrol	5 0 g
Propionic Acid	2 0 g
Oleic Acid	20 0 g
Wool Fat	7 5 g
Arachis Oil	to 100 0 ml

Mix the acids and 75 ml of the arachis oil add the dienœstrol and gently heat until dissolved Add the wool fat allow to cool and adjust to volume with the arachis oil

Œstradiol Ointment

Œstradiol Monobenzoate		0.1 g
Arachis Oil		10.0 g
Hydrous Wool Fat	to	100.0 g

Dissolve the œstradiol monobenzoate in the arachis oil with the aid of gentle heat and incorporate the solution in the hydrous wool fat

Œstrone ointment may be prepared in a similar manner

Stilbœstrol Cream

(a)	Stilbœstrol	5.0 g
	Oleic Acid	10.0 ml
	Wool Fat	7.5 g
	Arachis Oil	to 100.0 ml

Mix the oleic acid and 50 ml of the arachis oil add the stilbœstrol and gently heat until dissolved Add the wool fat, allow to cool and adjust to volume with arachis oil

(b)	Stilbœstrol	0.2 g
	Emulsifying Wax	10.0 g
	Arachis Oil	30.0 g
	Distilled Water	to 100.0 g

Melt the emulsifying wax in the arachis oil add the stilbœstrol and continue heating until solution is effected Allow to cool and add the distilled water in small portions shaking between each addition

Oily solutions are also used for percutaneous administration Deoxycortone acetate is sometimes given by rubbing a 0.02 per cent solution in benzyl alcohol into the skin of the thigh arm or abdomen

Vaginal and Rectal Administration Œstradiol œstrone and the artificial œstrogens are used as pessaries the basis being either oil of theobroma or glycerin suppository mass of the British Pharmacopœia The esters of the hormones are soluble in oil of theobroma and may be made in the usual way When glycerin suppository mass is used the hormones should be dissolved in propylene glycol before incorporation since they are not very soluble in water

The only hormone which appears to have been administered rectally is testosterone Suppositories of testosterone are used to introduce the hormone into the blood stream and not for a local effect They usually have an oil of theobroma base

Nasal Administration Œstrone has been used as a solution in arachis oil containing 100 micrograms in a millilitre for local nasal application[11] Stilbœstrol has been used similarly as a 0.1 per cent solution in arachis oil

Parenteral Administration The naturally occurring sex hormones are chiefly administered by intramuscular injection usually as the acetate benzoate or propionate For this purpose the esters are dissolved in a suitable oil such as arachis or olive oil or in ethyl oleate and the solution is sterilised by heating at 150 for one hour or if the volume of solution in a container exceeds 30 millilitres for a longer period of time sufficient to ensure that the whole of the contents are heated at 150 for one hour The following injections are described in the British Pharmacopœia

Injectio Œstradiolis Dipropionatis
Injectio Œstradiolis Monobenzoatis
Injectio Progesteroni
Injectio Testosteroni Propionatis

Deoxycortone acetate is also given intramuscularly in an oily solution prepared in a similar way (Injectio Deoxycortoni Acetatis B P) A water soluble glycosidic derivative of deoxycortone for intravenous injection is also available

Artificial œstrogens although usually given by mouth are sometimes administered by intramuscular injection Solutions in oil for this pur pose are prepared in a manner similar to that described for the natur ally occurring hormones

Aqueous solutions of natural and artificial hormones when required for injection are sterilised by heating in an autoclave Where the solu bility of the substance in water is very low a mixture of propylene glycol and water is often satisfactory as a solvent

Deoxycortone acetate is sometimes given by intravenous injection as a 0 1 per cent solution in propylene glycol the solution may be sterilised by heating at 150 for one hour in the final closed container

Certain sex hormones are administered by injection as a suspension of a microcrystalline powder in water When so injected the hormones are only slowly absorbed and therefore exert their effect over a pro longed period Cortisone is similarly used mainly as the acetate which is prepared in the form of microcrystals 5 to 10 microns in diameter and suspended in normal saline solution in a concentration of 25 milligrams per millilitre[1]

Implantation The most satisfactory method of obtaining a continuous and prolonged effect is by implanting a pellet of the hormone subcu taneously Pellets are supplied sterile in sealed glass tubes and the hormones usually administered in this way are œstradiol œstrone progesterone testosterone and deoxycortone acetate Methods of im implantation are described on page 161 Flat implantation pellets are sometimes preferred to fused pellets

Hormone Allergy Test Sets for diagnosis and for the desensitisation of patients with hormone allergy (see page 172) are available For diag nosis the sets contain solutions in olive oil of cholesterol deoxycortone acetate œstradiol monobenzoate œstrone progesterone and testos terone dipropionate in 0 5 millilitre ampoules containing 0 005 or 0 05 milligram together with ampoules of olive oil for control The sets for desensitisation consist of similar solutions containing 0 1 or 1 0 milligram per millilitre and are supplied in rubber capped bottles

Legal Requirements

In Great Britain a number of hormones and their preparations are subject to certain forms of legal control Insulin and pituitary (posterior

lobe) extracts are controlled by the Therapeutic Substances Act 1925 and insulin and the active principles of the pituitary suprarenal and thyroid glands together with the salts of the active principles of the suprarenal and thyroid glands are included in the Poisons List 1950 (S I 1950 No 1213) and various Schedules to the Poisons Rules 1949 (S I 1949 No 539)

Therapeutic Substances Act 1925 This act controls the manufacture sale and importation of those therapeutic substances the purity or potency of which cannot be adequately tested by chemical means The requirements of the Act are supplemented by regulations issued under the authority of the Act for hormones the most important regulations are the Therapeutic Substances Regulations 1931 (S R and O 1931 No 633) which define the general and special requirements for insulin and pituitary (posterior lobe) extracts These requirements specify *inter alia* the conditions under which the preparations must be manufactured the types of container the method of labelling and tests for strength quality and sterility

The containers for liquid preparations must be sealed glass containers made of non alkaline resistance glass and if multiple dose containers are used the preparation must contain a bacteriostatic to prevent the growth of any organism introduced during the removal of part of the contents

The label on the container must state the proper name of the substance as defined by the regulations the licence and batch number and the strength in units per millilitre the containers of insulin tablets must state the number of units in each tablet The label on the container or the label or wrapper on the package must state the name and address of the manufacturer the date on which the manufacture of the batch was completed the date up to which the preparation may be expected under suitable conditions to retain its potency the storage conditions necessary to retain this potency and the name and proportion of any added bacteriostatic Preparations may not be sold after the date of expiry stated on the label except to a medical practitioner who has been acquainted with this fact and is satisfied that the sale is required by the urgency of the case

Other special requirements for insulin forbid its issue in a mixture with any other therapeutic agent without the consent of the licensing authority and stipulate that if issued for injection as a suspension it shall be tested before suspension

Pharmacy and Poisons Act 1933 The following substances are included in Part I of the Poisons List

Insulin

Pituitary gland the active principles of

Suprarenal gland the active principles of their salts
Thyroid gland the active principles of their salts

These substances do not appear in the First or Fourth Schedule to the Poison Rules 1949 but are included in the Sixth and Seventh Schedules

The Sixth Schedule specifies a permissible form of disclosure for the proportion of the poison, and the relevant entries are as follows

Insulin The number of units of activity as defined in the British Pharmacopœia contained in a specified quantity of the preparation

Pituitary gland the active principles of Either
(*a*) The number of units of activity as defined in the British Pharmacopœia contained in a specified quantity of the preparation or
(*b*) the proportion of pituitary gland or of anterior or of posterior lobe of the gland as the case may be contained in the preparation or
(*c*) the amount of pituitary gland or of anterior or of posterior lobe of the gland as the case may be from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland substance

Suprarenal gland the active principles of their salts Either
(*a*) the proportion of suprarenal gland or of the cortex or of the medulla of the gland as the case may be contained in the preparation or
(*b*) the amount of suprarenal gland or of the cortex or of the medulla of the gland as the case may be from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland substance

Thyroid gland the active principles of their salts Either
(*a*) the proportion of thyroid gland contained in the preparation or
(*b*) the amount of thyroid gland from which a specified quantity of the preparation was obtained together with an indication whether th amount relates to fresh or to dried gland

The Seventh Schedule gives the special wording to be used on the labels of certain preparations in place of the word Poison Medicines made up ready for the internal treatment of human ailments must be labelled with the words Caution It is dangerous to take this preparation except under medical supervision if they contain one of the following

Insulin
Pituitary gland the active principles of
Thyroid gland the active principles of their salts

If they contain the active principles of suprarenal gland or their salts the prescribed wording is Caution It is dangerous to exceed the stated dose When the medicines are not made up ready for the internal treatment of human ailments the cautionary phrases given above do not apply and the word Poison must be used instead

When either of the cautionary phrases or the word Poison is used it must not be modified in meaning by adding any other words or marks and it must be either on a separate label or surrounded by a line within which there must be no words other than those required by law

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Suprarenal gland, the active principles of their salts
Thyroid gland the active principles of their salts

These substances do not appear in the First or Fourth Schedule to the Poison Rules 1949 but are included in the Sixth and Seventh Schedules

The Sixth Schedule specifies a permissible form of disclosure for the proportion of the poison and the relevant entries are as follows

Insulin The number of units of activity as defined in the British Pharmacopoeia contained in a specified quantity of the preparation

Pituitary gland the active principles of Either
(a) The number of units of activity as defined in the British Pharmacopoeia contained in a specified quantity of the preparation or
(b) the proportion of pituitary gland or of anterior or of posterior lobe of the gland as the case may be contained in the preparation or
(c) the amount of pituitary gland or of anterior or of posterior lobe of the gland as the case may be from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland substance

Suprarenal gland the active principles of their salts Either
(a) the proportion of suprarenal gland or of the cortex or of the medulla of the gland as the case may be contained in the preparation or
(b) the amount of suprarenal gland or of the cortex or of the medulla of the gland as the case may be from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland substance

Thyroid gland the active principles of their salts Either
(a) the proportion of thyroid gland contained in the preparation or
(b) the amount of thyroid gland from which a specified quantity of the preparation was obtained together with an indication whether the amount relates to fresh or to dried gland

The Seventh Schedule gives the special wording to be used on the labels of certain preparations in place of the word Poison Medicines made up ready for the internal treatment of human ailments must be labelled with the words Caution It is dangerous to take this preparation except under medical supervision if they contain one of the following

Insulin
Pituitary gland the active principles of
Thyroid gland the active principles of their salts

If they contain the active principles of suprarenal gland or their salts the prescribed wording is Caution It is dangerous to exceed the stated dose When the medicines are not made up ready for the internal treatment of human ailments the cautionary phrases given above do not apply and the word Poison must be used instead

When either of the cautionary phrases or the word Poison is used it must not be modified in meaning by adding any other words or marks and it must be either on a separate label or surrounded by a line within which there must be no words other than those required by law

REFERENCES

1 Leyton O and Poulton E P *Lancet* 1931 i 996
2 Bennett T I *Lancet* 1931 i 1053

Adreph ne Ampoules	1 ml ampoules contain ng adrenalin 1 in 2500 ephedrine hydrochloride 3 per cent and Chloretone 0 5 per c nt	Pa ke Dav s
Epl etonogen	1 ml ampoules conta ning adrenaline gr and eph drine hydrochloride ½ gr	Richt r
Ephetonogen Fo te	1 ml ampo les containing adrenaline $\frac{1}{60}$ gr ephed ne hydrochloride ¼ gr and atropine $\frac{1}{120}$ gr	Richter
Hyperduric Adrenalin	½ and 1 ml ampoules and 5 ml vials containing adrenaline 1 in 1000 as the mucate	All n and Hanburys
Pitalin	1 ml ampo iles containing inj c tion of pituitary (posterior lob) 0 5 ml and injection of adrenaline *0 36 ml*	Paines and Byrne
Pitrenalin	T vin ampoules contain ng 0 5 ml of p tuitrin and 0 36 ml of adrenalin chloride solution (1 in 1000)	Parke Davis
Suprarenalin	1 ml ampoules contain ng adrenaline 1 in 1000	Armour
Vas constrictine	1 ml ampoules and 30 ml v als conta ning adrenaline 1 in 1000	Duncan Flockhart

Ointments

Adrenal n and Chloretone Eye O ntment	Adrenal ne 1 in 4000 v ith Chloretone 5 per cent.	Parke Davis
Adr nalin and Chloreton O ntment	Adrenaline 1 in 1000 with Chloretone 5 per cent	Parke Davis
Adrenalin Ointment	Adrenaline 1 in 1000	Parke Davis
Anusan	Contains adrenal ne phenol and zinc oxide	Allen and Hanburys
Vaso-constrictine O ntm nt	Ad naline 1 in 1000	Duncan Flockhart

Sol tions

(Solut on of Adrenal ne B P conta ns adrenaline 1 in 1000 it is suppl ed in 10 ml vials and ½ 1 2 and 4 fl oz bottles)

Adrenal ne Borate Solut on	1 fl o bottles	British Drug Houses
Adreph ne	10 ml vial and 1 fl o bottles c ntaining adrenal ne 1 n 10 000 eph d ne sulphate 2 per cent and Chl etone 0 5 per cent	Parke Davis
Sup a nal n	1 fl oz and 1 l tre bottles contain ng adrenal ne 1 in 1000	Armour
Vaso-const ct ne	1 fl o bottles containing adrenalin 1 n 1000	Duncan Flockhart

S pp sit s

(Adren line Suppos to es B P C contain adrenal ne gr)

Adren l n and Chlor tone Supposito es	Adrenaline 1 n 1000 th Chlore tone 3 gr	Parke Dav s
Adrenalin Suppos to ies	Adren l n hydro hloride $\frac{1}{300}$ gr equ val nt to adrenal ne 1 n 1000	Parke Davis
Anu n	Conta n adrenal ne phenol and zinc oxide	Allen and Hanburys

CHAPTER VIII

COMMERCIAL PREPARATIONS

MOST of the hormones and related substances which have been described in earlier pages are obtainable in a variety of pharmaceutical forms sometimes under official or accepted names and sometimes under trade names The following list gives the names of a number of these preparations and the suppliers together with a brief description where necessary of the composition strength and type and size of container the full names and addresses of the suppliers will be found at the end of the chapter The list is not comprehensive and many of the preparations of the British Pharmacopœia and British Pharmaceutical Codex may be obtained by pharmacists from their usual drug suppliers under the official titles

The preparations are grouped according to the substance they consist of or contain the substances concerned being arranged alphabetically under the gland or part of gland to which they correspond the glands themselves are also arranged alphabetically

ADRENALS

Adrenaline

Inhalants

Preparation	Description	Supplier
Adrenaline Chloride Solution 1 in 100	5 ml vials	Parke Davis
Adrenalin Inhalant	1 fl oz bottles containing adrenaline 1 in 1000 with Chloretone	Parke Davis
Adrenaline Inhalant	5 and 10 ml bottles containing adrenaline 1 in 100	Burroughs Wellcome
Adrenaline Solution 1 in 100 (for inhalation)	5 ml and 1 fl oz vials 15 ml vials	Evans Oxo Paines and Byrne
Adrenaline Tartrate Spray Solution	10- and 25 ml bottles containing adrenaline 1 in 1000	Burroughs Wellcome
Adrephine Inhalant	10 ml vials containing adrenaline 1 in 10 000 ephedrine hydrochloride 1 per cent benzocaine 1 per cent and Chloretone 0 5 per cent in glycerin	Parke Davis

Injections

(Injection of Adrenaline B P contains adrenaline 1 in 1000 it is supplied in 0 5 1 and 2 ml ampoules and in 5 10 15 20 and 30 ml vials)

Preparation	Description	Supplier
Adrenalin Chloride Solution	0 5 and 1 ml ampoules 10 ml vial and 1 fl oz bottles containing adrenaline 1 in 1000 1 ml ampoules containing adrenaline 1 in 10 000	Parke Davis
Adrenalin in Oil	1 ml ampoules containing 2 mg	Parke Davis
Adrenaline Suspension with Ascorbic Acid	10 ml vials containing adrenaline 1 in 100	British Drug Houses
Adrenutol	Injectable suspension containing 1 or 2 mg per ml	Evans

Tabl ts
(Tablets of Methyltestosterone B P contain 5 mg unless oth rv se o dered)

Methyltestosterone	5 or 10mg	Boots
	5 10 25 or 50 mg	British Drug Houses Oxo
	5 mg	Burroughs Wellcome
Erugon S Tablets	5 mg	Baver
Glosso Sterandryl	5 10 25 or 50 mg	Rouss l
Neo Homb eol (M)	5 10 25 or 50 mg	O ganon
O av ron	5 10 25 or 50 mg	Priti h Schering
Perandren Linguets	5 10 25 or 50 mg	Ciba
Virormone Oral	5 10 25 o 50 mg	Paines and Byrne

Testosterone
Impl nt tion Tablets

Testosterone	25 50 100 150 200 300 or 350 mg	Organon

Injections

Virorm ne	Ampoules containing 5 10 25 50 100 or 250 mg	Paines and Byrne
V ro mone X	2 ml ampoul s conta n ng 50 mg n aqueous suspension	Pa nes and By ne

O ntm nt

Perand en	2 mg per g	Ciba
Virormone S lve	2 mg pe g	Paines and Byrne

Suppositories

Virormone	15 mg	Paines and Byrne

Testosterone Propionate
Implantation Tabl ts

Perandren Implants	100 mg	Ciba
Sterandryl Implant	100 mg	Roussel
Testosterone	100 mg	Br t sh Drug Hou
Propionate	25 50 100 150 200 300 or 350 mg	Organon

Inject o s
(Inject on of Testosterone Prop onate B P contains 10 mg per ml unless otherw se o dered)

Testo teron P op onate	1 ml ampoul s cont n ng 5 10 o 5 mg	Boots Purroughs Wellcome
	1 ml ampoules conta n ng 1 5 10 25 o 50 mg 2 ml ampoules containing 100 mg	Oxo
	1 ml ampoules onta n ng 5 10 25 o 50 mg 2 ml ampoul s con tain ng 100 mg 10 ml als con taining 500 mg	British Drug Houses
E ugon S	Ampoules conta n ng 5 10 or 25 mg	Baye
Neo-Hombreol	1 ml ampoules contain ng 5 10 5 or 50 mg 2 ml amp ules con ta n ng 100 mg 10 ml v als con taining 50 mg p r ml	Organon
Pantestin	Ampoules conta n ng 5 10 or 25 mg	R cht
Perandren	1 ml ampoules containing 5 10 25 o 50 mg 2 ml mpoules containing 100 mg 10 ml v als conta n ng 50 mg pe ml	C ba

Tablets

Adrenalin and Cocaine Hypodermic Tablets	Adrenaline 0 05 mg cocaine hydrochloride 0 01 g	Parke Davis
Adrenalin Hypodermic Tablets	0 3 or 1 mg	Parke Davis
Adrenaline Tablets	$\frac{1}{1000}$ or $\frac{1}{120}$ gr	Haines and Byrne
Suprarenalin	$\frac{1}{1000}$ or $\frac{1}{60}$ gr	Armour
Vaso constrictine Hypotabs	Adrenaline $\frac{1}{100}$ gr	Duncan Flockhart

Adrenal Cortex

Cortical Extracts

Cortigen	1 ml ampoules and 10 and 30 ml vials	Richter
Eschatin	10 ml vials containing 200 μg of cortisone per ml	Parke Davis
Eucortone	10 ml vials	Allen and Hanburys
Supracort	Each ml is equivalent to 75 g of fresh gland 1 ml ampoules and 10 ml vials	Haines and Byrne
Suprarenal Cortex Extract	*10 ml vials*	*British Drug Houses*

Deoxycortone Acetate

Implantation Tablets

D O C A	25 50 100 150 200 or 300 mg	Organon
Deoxycortone Acetate	100 mg	British Drug Houses
Percorten Implants	100 mg	Ciba
Syncortyl Implants	100 mg	Roussel

Injections

(Injection of Deoxycorton Acetate B P contains 5 mg per ml unless otherwise ordered)

Deoxycortone Acetate	1 ml ampoules containing 5 or 10 mg 10 ml vials containing 10 mg per ml	British Drug Houses
Cortiron	1 ml ampoules containing 2 5 or *10 mg*	British Schering
D O C A	1 ml ampoules containing 2 5 or 10 mg 5 ml vials containing 5 mg per ml	Organon
Percorten	1 ml ampoules containing 2 5 or 10 mg 10 ml vial containing 10 mg per ml	Ciba
Percorten Crystules	2 ml ampoules containing 50 mg in aqueous suspension with 2 mg of Nupercaine	Ciba
Percorten Water Soluble	5 ml ampoules containing deoxycortone glucoside in aqueous solution	Ciba
Syncortyl	1 ml ampoules containing 5 or 10 mg	Roussel

Sublingual Tablets

D O C A	*1 mg*	Organon
Percorten Linguets	1 mg	Ciba

Gonads

Androgens

Methyltestosterone

Injection

Micryston Methyltestosterone	8 ml vials containing 5 mg per ml suspended in normal saline	Coates and Cooper

Hexœstrol

I j tions

Hexœstrol	1 ml ampoules contain ng 1 or 5 mg	Boots; B tish Drug Houses; Paines and Byrne

Tabl ts

(Tablets of Hexœstrol B P contain 1 mg unless otherwise ordered)

Hexœstrol	1 or 5 mg	Boots; B rroughs Wellcome
	0 5 1 or 5 mg	British Drug Houses; Oxo
	0 1 0 5 1 o 5 mg	Organon; Paines and Byrne
Hexital	3 mg with 20 mg of phenoba b tone	Ortho Pharmaceuti cals
Thelestrol	3 mg with 20 mg of phenobarbi tone	Carnrick

Hexœstrol Dipropionate

Inject n

Hexœst ol D p opio-nate	1 5-ml ampoules containing 15 mg	Burroughs Wellcome

Œstradiol

Implantat on Tablets

Gynœstryl Implants	20 mg	Roussel
Œstrad ol Implanta tion Pellets	10 15 20 25 50 or 100 mg	Organon
Œstroform Pellets	20 mg	B t sh Drug Houses
Ovocyclin Implants	20 mg	Ciba

Ointment

Ovocyclin	*0 1 mg per g*	C ba
Progynon	0 1 mg pe g	British S I ring

P ssaries

P ogynon Vaginal Suppositories	0 36 mg	British Sch ring

Subl ngual Tabl t

Ovocycl n Linguets	0 04 0 1 o 1 mg	C ba

Tabl ts

Glandubol n	0 1 0 5 or 1 mg	Richter

Œstradiol Dipropionate

Inj t ons

(Injection of Œstrad ol Dip opionate B P contains 1 mg pe ml unless others se ordered.)

Dimenformon Dip o-pionate	5-ml v als conta n ng 25 mg in oil	Organon
Glandubol n	1 ml ampoules conta ning 0 1 1 o 5 mg	Richter
Ovocycl n P	1 ml ampoules contain ng 1 o 5 mg n o l	Ciba
P ogynon DP	1 ml ampoules contain ng 20 000 i b u *	Br tish S I ering

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I t m t nal benzoat nit

Perandren Crystules	2 ml ampoules containing 50 mg in aqueous suspension with 2 mg of Nupercaine	Ciba
Sterandryl	1 ml ampoules containing 5 10 25 or 50 mg 2 ml ampoules containing 100 mg 10 ml vials containing 500 mg	Roussel
Testoviron	1 ml ampoules containing 5 10 or 25 mg	British Schering
Valotest	1 ml ampoules containing 5 10 or 25 mg	Wallace
Ointments		
Neo Hombreol	2 or 25 mg per g	Organon
Perandren	2 mg per g	Ciba
Testosterone Ointment	2 5 per cent	Boots
Testoviron	2 mg per g	British Schering
Suppositories		
Neo-Hombreol	15 mg	Organon

Œstrogens

Dienœstrol

Ointment

Dienœstrol Ointment	2 5 per cent	British Drug Houses

Tablets

(Tablets of Dienœstrol B P contain 0 1 mg unless otherwise ordered)

Dienœstrol	0 1 0 3 1 or 5 mg	Allen and Hanburys Boots British Drug Houses Oxo Paines and Byrne
	0 03 0 1 0 3 1 or 5 mg	Organon
Armo Nœstrol	0 1 mg with 16 mg of phenobarbitone	Armour
Armo-Nœstrol Forte	0 3 mg with 16 mg of phenobarbitone	Armour

Ethinylœstradiol

Elixir

Estigyn	0 02 mg per 60 ml	British Drug Houses

Tablets

(Tablets of Ethinylœstradiol B P contain 0 02 mg unless otherwise ordered)

Amenorone	0 01 mg with 10 mg of ethisterone	Roussel
Barbœstrol	0 01 mg with 16 mg of phenobarbitone and of papaverine	Roussel
Estigyn	0 01 0 05 or 1 mg	British Drug Houses
Ethidol	0 01 or 0 05 mg	British Schering
Ethin Œstryl	0 01 0 05 or 1 mg	Roussel
Ethinylœstradiol	0 01 or 0 05 mg	Armour
	0 01 0 05 0 1 or 1 mg	Oxo
	0 01 0 05 or 1 mg	Paines and Byrne
Eticyclin	1 mg	Ciba
Eticyclin Linguets	0 01 or 0 05 mg	Ciba
Lynoral	0 01 0 05 or 1 mg	Organon
Orasecron	0 05 mg with 10 mg of ethisterone	British Schering

Ointments		
Menformon	0 5 mg per g	Organon
Œstroform	1000 i u per g	British Drug Houses
Œstrosal e	10 000 i u per oz	Paines and Byrne
Œstrosalve Concentrated	2500 i u per g	Paines and Byrne
Unden	5000 i u per g	Bayer
Pessari s		
Kolpon Vaginal Bougies	0 1 mg	Organon
Œstroform	1000 i u (adult or child size)	British Drug Houses
Œstrone	1000 or 5000 i u	Paines and Byrne
Sol t		
Menformon	10-ml bottles containing 1 mg per ml for oral use	Organon
Oleum Ketodestrin	O ly solut on containing 5000 i u per g	Pa nes and Byrne
Sol Ketodestrin	1-oz bottles of aqueous solut on containing 1000 i u per 60 m	Pa nes and Byrne
Solestrin	15- and 30-ml bottles of alcoholic solut on conta n ng about 1300 i u per drop	Paines and Byrne
S ppo itories		
Menformon	0 1 or 1 mg	Organon
T blets		
(Tablets of Œstrone B P contain 1 mg unless otherwise ordered)		
Menformon	0 05 0 1 0 3 1 o 5 mg	Organon
Œstrin	1000 i u	Oxo
Œstroform	1000 5000 or 10 000 u	Briti h Drug Houses
Œstrone	500 1000 3000 5000 or 10 000 i u	Paines and Byrne
Progynon	1000 3000 or 10 000 i u	British Schering
Strog ne	1 mg of conjug ted œstrogens calculated as sodium œstrone sulphate	Wyeth
Unden Pellets	100 500 or 1000 u	Bayer

Stilbœstrol

Inject		
Pabest ol	1 ml ampoules containing 1 or 5 mg	Paines and Byrne
St lbœst ol	1 ml ampoules contain ng 1 o 5 mg	Allen and Hanburys; Boots; British Drug Houses
	1 ml ampoules conta ning 5 ml	Burroughs Wellcome
	0 5-ml ampoules containing 0 5 mg 1 ml ampoules conta ning 1 or 5 mg	Crookes
O ntments		
Pabestrosal e	O ntm nt conta ning 0 2 per cent with benzoca ne 2 5 per cent	Paines and Byrne
Stilbœstrol Ointment	0 5 or 1 per cent	Boots
Stilbœstrol Salve	0 1 mg per g	Crookes
Pess ies		
Stilbœstrol and Lac t c Acid	Stilbœstrol 1 mg with lactic ac d 1 per cent	Crookes
Sp ay		
Stilbœstrol Sp y	0 5 mg per ml in oil	Crookes

Œstradiol Monobenzoate

Injections

(Injection of Œstradiol Monobenzoate B P contains 1 mg per ml unless otherwise ordered)

Benzo Gynœstryl	1 ml ampoules containing 1 or 5 mg	Roussel
Benztrone	1 ml ampoules containing 1 2 or 5 mg	Paines and Byrne
Disecron	1 ml ampoules and 10 ml vials containing 2 5 mg with 12 5 mg of progesterone per ml	British Schering
Dimenformon	1 ml ampoules and 5 ml vials containing 10 000 i b u per ml 1 and 2 ml ampoules and 5 and 10 ml vials containing 50 000 i b u per ml	Organon
Gynformone	1 ml ampoules containing 10 000 i b u	Wallace
Gynformone Forte	1 ml ampoules containing 50 000 i b	Wallace
Œstrin	1 ml ampoules containing 1000 10 000 20 000 or 50 000 i b u	Oxo
Œstroform	1 ml ampoules containing 1000 10 000 20 000 or 50 000 i b u	British Drug Houses
Ovocyclin B	1 ml ampoules containing 1 or 5 mg in oil	Ciba
Ovocyclin B Crystules	20 ml ampoules containing 10 mg in aqueous suspension with 2 mg of Nupercaine	Ciba
Progynon B Oleosum	1 ml ampoules and 5 and 10 ml vials containing 10 000 or 50 000 i b u per ml	British Schering
Unden	1 ml ampoules and 10-ml bottles containing 10 000 i b u per ml ml ampoules containing 50 000 i b u	Bayer

Ointment

Dimenformon	2 mg per g	Organon

Œstriol

Capsules

Theelol	0 12 mg	Parke Davis

Œstrone

Injections

Ketodestrin	1 ml ampoule containing 0 05 0 1 0 5 and 1 mg	Paines and Byrne
Menformon	1 ml ampoules containing 0 1 mg (aqueous) 1 ml ampoules and 5 ml vials containing 1 mg per ml in an organic solvent	Organon
Micryston Œstrone	8 ml vial containing 1 mg per ml suspended in normal saline	Coates and Cooper
Theelin (Aqueous Suspension)	1 ml ampoules containing 2 mg	Parke Davis
Theelin in Oil	1 ml ampoules containing 0 1 0 2 0 5 or 1 mg	Parke Davis
Unden	1 ml ampoules containing 1000 i u in aqueous solution	Bayer

Injections		
Corpus Luteum Extract	5-ml ampoules and 10- and 20-ml vials (1 ml is equivalent to about 3 gr of dried gland)	Armour
	0 5- and 1 ml ampoules and 10- and 20-ml bottles (1 ml is equivalent to 2 gr of dried gland)	Oxo
	1 ml ampoules containing the equivalent of 1 g of fresh gland	Paines and Byrne
Tablets		
Corpus Luteum	½ 1 2 or 5 gr	Armour
	1 2 or 5 gr	Paines and Byrne
Corpus Luteum Substance Glanules	0 1296 g of dried gland	Armour
Luteomensin	Tablets containing ¼ or ½ gr of extract	Paines and Byrne

Ethisterone

(Tablets of Ethisterone B P contain 5 mg unless otherwise ordered)

Ethisterone	5 or 10 mg	Boots
	5 mg	Burroughs Wellcome
	5 10 or 25 mg	British Drug Houses
Gestone Oral	5 10 or 25 mg	Paines and Byrne
Lutocyclin Linguets	5 10 or 25 mg	Ciba
Lutogyl Oral	5 10 or 25 mg	Roussel
Oraluton	5 10 or 25 mg	British Schering
Progestoral	5 10 or 25 mg	Organon

Progesterone

Implantation Tablets		
Lutocyclin Implants	100 mg	Ciba
Lutogyl Implants	100 mg	Roussel

Injections

(Injection of Progesterone B P contains 5 mg per ml unless otherwise ordered)

Progesterone	1 ml ampoules containing 2 5 or 10 mg	Boots Burroughs Wellcome
	1 ml ampoules containing 1 2 5 10 25 or 50 mg	Oxo
	1 ml ampoules containing 5 or 10 mg 10-ml vials containing 5 mg per ml	Parke Davis
Duecron	1 ml ampoules and 10-ml vials containing 12 5 mg with 2 5 mg of oestradiol monobenzoate per ml	British Schering
Gestone	1 ml ampoules containing 1 2 5 10 or 25 mg	Paines and Byrne
Glanducorpin	2 5- or 10-mg ampoules	Richter
Gynoluton	1 ml ampoules containing 2 mg	Wallace
Gynolutone Forte	1 ml ampoules containing 5 mg	Wallace
Luteomensin	1 ml ampoules containing 1 mg	Paines and Byrne
Lutocyclin	1 ml ampoules containing 2 5 10 or 25 mg 10-ml vials containing 25 mg per ml	Ciba
Lutocyclin Crystules	2 ml ampoules containing 50 mg with 2 mg of Nupercaine	Ciba
Lutogyl	1 ml ampoules containing 2 5 or 10 mg	Roussel
Lutren	1 ml ampoules containing 2 or 5 mg	Bayer

Tablets

(Tablets of Stilbœstrol B P contain 0 5 mg unless otherwise ordered)

Preparation	Form / Strength	Manufacturer
Stilbœstrol Tablets	0 5 1 or 5 mg	Allen and Hanburys British Drug Houses Burroughs Wellcome Oxo
	0 1 0 5 1 or 5 mg	Boots Organon
	0 1 or 5 mg	Crookes
Enseals Diethylstilbœstrol	0 5 or 1 mg	Lilly
Ovendosyn	Tablets containing 0 5 mg with calcium phosphate 200 mg	Menley and James
Ovendosyn Forte	Tablets containing 5 mg with calcium phosphate 325 mg	Menley and James
Pabestrol	0 05 0 1 0 5 1 or 5 mg	Paines and Byrne
Pabestrol EC	Enteric coated tablets containing 0 5 1 or 5 mg	Paines and Byrne
Sedestrol	Tablets containing 0 1 mg with 16 mg of phenobarbitone	Geigy
Stilbœstrol Compound	Tablets containing 1 mg with 16 mg of phenobarbitone	Boots

Stilbœstrol Dipropionate

Injections

Preparation	Form / Strength	Manufacturer
Pabestrol D	1 ml ampoules containing 1 or 5 mg	Paines and Byrne
Stilbœstrol Dipropionate	1 ml ampoules containing 1 or 5 mg	Boots British Drug Houses Organon
	0 5-ml ampoules containing 0 5 mg 1 ml ampoules containing 1 or 5 mg	Crookes
	Oily solution ampoules containing 1 or 5 mg 25 ml bottles containing 10 mg	Oxo
Syntestrin	Ampoules containing 1 or 5 mg	Richter

Ointment

Preparation	Form / Strength	Manufacturer
Stilbofax	0 05 per cent	Burroughs Wellcome
Syntestrin	30 mg per oz	Richter

Suppositories

Preparation	Form / Strength	Manufacturer
Syntestrin	1 mg	Richter

Tablets

Preparation	Form / Strength	Manufacturer
Pabestrol D	0 1 0 5 1 or 5 mg	Paines and Byrne
Stilbœstrol Dipropionate	0 1 0 5 1 or 5 mg	Boots
	0 5 1 or 5 mg	British Drug Houses
	0 1 1 or 5 mg	Crookes
Syntestrin	0 1 0 25 0 5 1 or 5 mg	Richter

Triphenylethylene Derivatives

Preparation	Form / Strength	Manufacturer
Gynosone	Tablets containing 0 5 g of triphenylchloroethylene	Imperial Chemical (Pharmaceuticals)

Progestogens

Corpus Luteum

Preparation	Form / Strength	Manufacturer
Corpus Luteum Powder	½ 1 or 4 oz	Armour

Capsules

Preparation	Form / Strength	Manufacturer
Corpus Luteum	2 5 or 10 gr	Armour

Miscellaneous

Ambinon A	1 ml ampoules containing the equivalent of 100 to 300 guinea pig units of thyrotrophic hormone and about 10 synergic rat units of gonadotrophic hormone supplied with ampoules containing 100 units of chorionic gonadotrophin	Organon
Ambinon B	Similar to Ambinon A but without the chorionic gonadotrophin	Organon
Antephysan	1 ml ampoules of extract	Richter
Anterior Pituitary Extract	1 ml ampoules	Parke Davis
Antoxylin	0.5- and 1 ml ampoules and 10- and 20-ml vials containing the equivalent of 2 gr of dried gland per ml	Oxo
Pitexan Snuff	Anterior lobe powder	Paines and Byrne
Pituitary Anterior Lobe	1 ml ampoules and 10- and 20-ml vials containing the equivalent of 1½ gr of dried gland	Armour
	1 ml ampoules containing the equivalent of 2 g of fresh gland	Paines and Byrne
Synapoidin	10-ml vials containing a combination of the follicle stimulating hormone from the anterior lobe and chorionic gonadotrophin contains 15 synergic rat units per ml	Parke Davis

Gonadotrophins from Other Sources

Chorionic Gonadotrophin

Antoxylin (S)	Ampoules containing 100 or 500 i u	Oxo
Antuitrin S (Antrodin)	10-ml vials containing 100 i u per ml 5 ml vials containing 500 i u per ml	Parke Davis
Glanduantin	Ampoules containing 100 or 500 units	Richter
Gonadotraphon L H	Ampoules containing 100 500 1000 or 1500 i u	Paines and Byrne
Gonadyl Chorionic	Ampoules containing 1000 i u	Roussel
Gonan	Ampoules containing 100 or 500 i u	British Drug Houses
Physostab	Ampoules containing 500 or 1000 i u	Boots
Pregnyl	Ampoules containing 100 500 or 1500 i u	Organon
Prolan	Ampoules containing 100 500 or 2000 i u	Bayer
Synapoidin	10-ml vials containing a combination of the follicle stimulating hormone from the anterior lobe and chorionic gonadotrophin contains 15 synergic rat units per ml	Parke Davis

Serum Gonadotrophin

Antostab	Ampoules containing 500 or 1500 i u	Boots
Gestyl	Ampoules containing 200 400 1000 or 3000 i u	Organon
Gonadotraphon F S H	Ampoules containing 400 1000 1500 or 3000 i u	Paines and Byrne

Micryston Progesterone	8 ml vials containing 12 5 mg per ml suspended in normal saline	Coates and Cooper
Progestin	1 ml ampoules containing 1 2 5 10 or 25 mg 10 ml vials containing 25 mg per ml	British Drug Houses
	1 ml ampoules containing 1 2 5 10 or 25 mg 5 ml vials containing 2 5 or 10 mg per ml 10 ml vials containing 1 or 25 mg per ml	Organon
Proluton	1 ml ampoules containing 2 5 10 or 25 mg	British Schering

Pancreas

Injection of Insulin

Insulin	20 40 or 80 units per ml	Allen and Hanburys Boots British Drug Houses
Unmodified Insulin	20 40 or 80 units per ml	Burrough Wellcome

Injection of Globin Zinc Insulin

Globin Insulin (with Zinc)	40 or 80 units per ml	Allen and Hanburys Boot British Drug Houses Burroughs Wellcome

Injection of Protamine Zinc Insulin

Protamine Zinc Insulin	40 or 80 units per ml	Allen and Hanburys Boots British Drug Houses Burroughs Wellcom

Other Insulin Preparations

Delay Insulin A B	Protamine insulin with solution of sodium phosphate to be added immediately before use 40 and 80 units per ml	British Drug Houses
Insulin Ointment	1 or 10 units per g	Organon

Parathyroids

Dihydrotachysterol

A T 10	15 ml vials containing 1 25 mg per ml in oil Capsules containing 0 625 mg in oil	Bayer

Parathyroid Extract

Para thormone	5 ml vials containing 20 units per ml	Lilly
Paroidin	5 ml vials containing 100 units per ml	Parke Davis
Parathyroid Hormone	5 ml vials containing 20 units per ml	Paines and Byrne

Pituitary

Anterior Lobe

Adrenocorticotrophic Hormone

Cortrophin	—	Organon

Growth Hormone

Antuitrin Growth	20 ml vials containing 8 rat growth units per ml	Parke Davis

Methylthiourac l	50- 100 or 200-mg tablets	Br tish Drug Houses Imperial Chemical (Pharmaceut cal)
	50- or 200 mg tablets	Organon
	100 or 200 mg tablets	Burroughs Wellcome

(Tablets of Methylt ouracil B P conta n 0 1 g unless otherwise ordered)

Propylthiouracil	25 mg tablets	Genatosan

(Tablets of Propylthiouracil B P contain 25 mg unless otherwise ordered)

Thyroxine and Related Substances

D odotyrosine	100-mg tablets	Organon
	¼ and 1¼ gr tablets	Paine and Byrne
Eltyran Tablets	0 15 mg organ c iodine and 0 09 mg thyroxine equivalent to 90 mg of thyroid	Bayer
Thyroglobulin Tablets	¼ gr	Oxo
Thyroxine	Tabl ts conta ning 0 3 or 1 mg of DL thyroxine	Brit sh Scher ng
	Tablets containing 0 2 or 0 8 mg of DL thyrox ne sodium hypoder m c tablets 1 mg	B tish Drug Houses
Thyroxine sod um	1 g or 10-g bottles	Burroughs Wellcome
L Thyroxine sod um	Tablets contain ng 0 05 or 1 mg of L thyroxine sod um	Glaxo

Whole Gland

Thyroid is suppl ed in tablets ranging in content f om $\frac{1}{10}$ gr to 5 gr by various manufacturers

Liquor Thyroidei	Represents 20 per cent of fresh substance	Oppenhe mer
Paromin	Enteric coated tablets conta ning $\frac{1}{160}$ gr of organ cally comb ned iodine and ½ gr of thyroid	Paines and Byrne
Thyranon	Tabl ts contain ng ½ 1 1½ or 2 gr	O ganon
Thy ocol	Capsules conta n ng active colloid equ valent to 5 gr of thyro d	Oppenhe mer

MANUFACTURERS

The full names and addres es of the manufactu s of th p eparations in the abo list a e as follows

Allen and Hanburys Ltd Bethnal Green London E 2
Armour Laboratories Ltd L ndsey St t London E C 1
Bayer P oducts Ltd Africa Hous K ngsway London W C 2
Boots Pure D ug Co Ltd Stat on Street Nott ngham
B t sh Drug Houses Ltd G aham Street C ty Road London N 1
Brit h Sche ng Ltd 229–231 Ken ngton High Stre t London W 8
Burroughs Wellcome and Co 183–193 Euston Road London N W 1
G W Carnrick and Co Newa k New Jersey (Distributors Brooks and Warburton Ltd 240 Vauxhall B dge Road London S W 1)
C ba Laboratories Ltd Horsham Sus x
Coates and Coope Ltd Py m d Works West Drayton M ddlesex
C ookes Laboratories Ltd Pa k Royal London N W 10
Duncan Flockhart and Co Ltd 104–108 H lyrood Road Ed nbu gh
Evans Medical Suppl es Ltd Speke Liverpool 19
Ged on Richte (G eat Br t in) Ltd W d ngton Road London N W 5
Genatosan Ltd Loughborough Le cestershire
Glaxo L borato es Ltd Greenford M ddlesex
Imperial Chem cal (Pharmac ut cals) Ltd Ful haw Hall Wilmslo v Manchester
Lilly and Co Ltd Bas ngstoke H nts
Menley and James Ltd 119–123 Coldharbour Lan London S E 5

Gonadyl Seric	Ampoules containing 400 or 1000 i u	Roussel
Luteoantin	Ampoules containing 100 or 400 units	Richter
Serogan	Ampoules containing 200 or 1000 i u	British Drug Houses

Posterior Lobe

Injection of Oxytocin

(Injection of Oxytocin B P contains 10 units (oxytocic) per ml)

Pitocin	0 5 and 1 ml ampoules and 5 ml vials	Parke Davis

Injection of Pituitary (Posterior Lobe)

(Injection of Pituitary (Posterior Lobe) B P contains 10 units (oxytocic) per ml)

Glanduitrin	0 5 and 1 ml ampoules and 10-ml vials	Richter
Infundibulin	0 5 and 1 ml ampoules and 10- and 25 ml vials	Evans
Infundin	0 5 and 1 ml ampoules	Burroughs Wellcome
Pitibulin	0 5 and 1 ml ampoules	Allen and Hanburys
Pituitary (Posterior Lobe) Extract	0 5 and 1 ml ampoules and 10- and 20-ml vials	Armour
	0 5 and 1 ml ampoules	Boots
	0 5 and 1 ml ampoules and 15 ml vials	British Drug Houses
	0 5 and 1 ml ampoules	Paines and Byrne
Pituitrin	0 5 and 1 ml ampoules and 10-ml vials	Parke Davis

Injection of Vasopressin

The B P injection contains 10 units per ml The commercial preparation most nearly resembling it is the following

Pitressin	0 5 and 1 ml ampoules containing 20 pressor units per ml	Parke Davis

Snuffs

Disipidin	100 gr bottles or 1½ gr capsules	Paines and Byrne
Glanduitrin	500 i u per g	Richter
Piton Powder	500 i u per g in lactose	Organon
Pituitary (Posterior Lobe) Snuff	¾ gr capsules	Armour

Other Posterior Lobe Preparations

Pitoxylin	Extract of the posterior lobe supplied in 0 5 ml ampoules containing 5 units per ml 0 5 and 1 ml ampoules and 10-ml bottles containing 10 units per ml and 10 ml bottles containing 20 units per ml	Oxo
Pitressin Tannate in Oil	1 ml ampoules containing 5 pressor units	Parke Davis

Thyroid

Antithyroid Substances

Thiouracil	50 100 or 200 mg tablets	British Drug Houses; Imperial Chemical (Pharmaceuticals)
	50- or 200-mg tablets	Organon

(Tablets of Thiouracil B P contain 0 1 g unless otherwise ordered)

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